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Introduction to the Northwest Ontario Lake Size Series (NOLSS)¹

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Fee, E. J., and R. E. Hecky. 1992. Introduction to the Northwest Ontario Lake Size Series (NOLSS). *Can. J. Fish. Aquat. Sci.* 49: 2434–2444.

The rationale, design, and limitations of the Northwest Ontario Lake Size Series (NOLSS) research program are described. The primary purpose of NOLSS is to discover how lake size per se influences limnological and fisheries phenomena, so that conclusions drawn from studies of particular lakes can be rigorously scaled and applied to lakes of other sizes. NOLSS consists of six lakes located in a remote wilderness region of Northwest Ontario. These lakes were chosen for their geological, hydrological, and morphological similarity (Canadian Shield geology; water renewal time >5 yr; fully stratified in summer), but they form an exponential gradient in surface area (from 89 to 34 700 ha.) Associated with this gradient of lake size are gradients of physical properties (turbulent energy, mixing depth, thermal behaviour) to which biological communities must adapt. NOLSS fills the conspicuous gap in size that separates two well-studied groups of lakes in Northwest Ontario: the Experimental Lakes Area (ELA), where whole-lake manipulation experiments are performed, and the Laurentian Great Lakes (Nipigon, Superior), where these experiments find some of their most important applications.

We are moving beyond a period of acute, localized, and relatively simple environmental problems to one of chronic, global, and extremely complex problems.

—WATER 2020, Science Council of Canada (1988)

L'étude décrit la justification, l'objet et les limites du programme de recherche NOLSS (Northwest Ontario Lake Size Series). Le but premier de la NOLSS est de déterminer de quelle façon la taille des lacs influe en elle-même sur les phénomènes limnologiques et halieutiques, de façon à ce que les conclusions tirées des études menées sur des lacs particuliers puissent être rigoureusement adaptées et appliquées à des lacs de taille différente. La NOLSS est basée sur six lacs situés dans une région sauvage éloignée du nord-ouest de l'Ontario. Ces lacs ont été choisis pour leur similarité géologique, hydrologique et morphologique (géologie du Bouclier Canadien; temps de renouvellement des eaux >5 ans; entièrement stratifiés en été), mais ils forment un gradient exponentiel de la surface (de 89 à 34 700 ha). À ce gradient de taille des lacs sont associés des gradients de propriétés physiques (énergie de turbulence, profondeur de mélange, comportement thermique) auxquels les communautés biologiques doivent s'adapter. La NOLSS comble la différence apparente de taille qui sépare les deux groupes de lacs bien étudiés du nord-ouest de l'Ontario: l'ELA (Experimental Lakes Area), où l'on procède à des expériences de manipulation de l'ensemble des lacs, et les Grands Lacs Laurentiens (Nipigon, Supérieur), où certaines des applications les plus importantes de ces expériences sont mises en oeuvre.

Nous sortons d'une période de problèmes environnementaux aigus, localisés et relativement simples pour aborder une ère de problèmes chroniques, globaux et extrêmement complexes.

—EAU 2020, Conseil des sciences du Canada (1988)

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Public awareness of the detrimental effects of human activities on natural ecosystems is a surprisingly recent development. Prior to 1960, it was rare to hear about the "environment," while today the news media report frequently and extensively about ecological problems (eutrophication, acid rain, desertification, deforestation, global warming, introduction of foreign species, urban air pollution, stratospheric ozone depletion, oil spills, destruction of tropical rain forests, contamination of aquifers, and bioaccumulation of toxic chemicals such as mercury and PCBs in food chains). The best evidence of how far we have come in only 30 yr is that public opinion

surveys taken today consistently place the "environment" at or near the top of the list of critical problems facing society.

The nature of ecological research has also changed dramatically during the last 30 yr. Before 1960, there were few freshwater ecologists (limnologists), and even fewer of them considered whole ecosystems — the lake together with its watershed and airshed — to be a manageable research subject. Fortunately, the first major environmental problem that they were called upon to solve (eutrophication) proved to be an ideal subject for whole-ecosystem research: because the source of the problem (excess nutrient loading) was always local, and the effects (excess algal biomass) were dramatic and immediate, whole-ecosystem experiments (Schindler 1977) could easily and dramatically demonstrate important causal linkages. Understanding the next major environmental problem (acidification)

¹This is the first in a series of papers describing work of the Freshwater Institute Science Laboratory on a lake size series in Northwest Ontario.

was not so simple. Here the source of the problem (acid rain) was diffuse and usually distant from affected lakes, and effects (loss of biodiversity in lower trophic levels, reproductive failures of fish) developed slowly and were subtle. Consequently, a long time period was required simply to identify the problem, and elucidating precisely how acid disrupted natural processes in lakes required both multiyear, whole-ecosystem experiments (Schindler et al. 1985) and coordinated data gathering over wide areas and long time periods.

We are now moving into an era when global problems (atmospheric warming, stratospheric ozone depletion, atmospheric transport of toxic chemicals) dominate the environmental agenda. Understanding how these will affect freshwater ecosystems will be even more difficult than was the case for acid rain, and current research infrastructures may not be able to respond effectively to the challenges that such problems pose. The most obvious of these challenges is to unambiguously distinguish their initially weak effects from inherent natural variability. Another will be to develop site-specific management strategies for the enormous number of lakes that will be affected without being able to undertake site-specific research. We believe that the information required to effectively address these and other pressing ecological mysteries can be obtained from the comparative study of a series of pristine lakes that span a wide range of sizes. Ideally, the lakes in this series would be located in a geologically, hydrologically, and meteorologically uniform wilderness region far from any significant point source of contamination, a wide range of lake sizes would be included, study method would be rigidly defined and adhered to for the lifetime of the study, and the study would extend for a long enough time period that size-related differences of interannual natural variability could be identified.

This paper introduces the Northwest Ontario Lake Size Series (NOLSS) program, which addresses these criteria. Our purpose here is to identify gaps in the current research infrastructure that NOLSS fills, to specify the hypotheses underpinning NOLSS, to explain the NOLSS experimental design, and to present basic limnological information about specific lakes included in NOLSS.

Canada is blessed with a disproportionate share of the world's surficial freshwater — 10% of the world's total renewable supply and over 50% of the world's lake surface area. The country encompasses very broad ranges of geology, hydrology, and climate, and interactions of these physical factors have created a great variety of aquatic habitats. Until more is known about the specific effects of lake size — and in particular, its relationship to natural variability — development of effective general models of these valuable ecosystems will not be possible. Without such models, aquatic ecologists will be unable to anticipate the impacts of the increasingly complex environmental problems of the future. In turn, Canada may have a unique role (and responsibility) in the elucidation of these effects because its wealth of pristine lakes provides the necessary raw material needed to address these issues empirically.

Effects of Lake Size

A few studies have directly addressed the issue of how lake size affects the structure of aquatic ecosystems (Colinvaux and Steinitz 1980; Patalas 1984; Matuszek and Beggs 1988; Gorham and Boyce 1989; Sterner 1990). In these studies, single measurements were taken from a large number of lakes of different sizes and then analyzed for statistical trends. Because any single sample from a lake can give very misleading information

about mean conditions, and because the lakes included in these studies were not carefully screened to ensure that size was the only important variable, all of these papers leave a tremendous amount of variance unexplained. Moreover, this approach can only identify size-related differences that exist at the time of the year when samples are taken (usually midsummer).

Figure 1 illustrates that size (surface area) plays a dominant role in determining the physical structure of lakes at all times of the year. Included are data from a small Experimental Lakes Area (ELA) lake, the NOLSS lakes, and Lakes Nipigon and Superior; all these lakes are located on the Canadian Shield in Northwest Ontario, experience the same climate, are deep enough to exhibit full thermal stratification during the summer, and have water renewal times in excess of 5 yr. The most dramatic size-related temperature differences are in early June (top panel), when the smallest (ELA) lake is fully stratified at 20°C, Lake Superior is unstratified at a temperature of 2.8°C, and intermediate-sized lakes fall between these two extremes in accordance with their sizes. The middle panel illustrates that larger lakes have lower maximum summer temperatures, achieve these maxima later in the summer, heat more slowly in the spring, and have a shorter period of stable stratification. The data in the bottom panel show that the mixed layer is deeper and much more variable over time in the large lakes. Another important size-dependent physical difference (not shown in the figure) is that ice-cover remains later in the spring and reforms later in the autumn in large lakes. For example, in 1990, ice disappeared from ELA L.373 on April 26 and reformed on November 19, while the corresponding events on Lake Nipigon occurred about a month later (May 25 and December 27).

While these physical differences are logical and fairly easy to demonstrate, their ecological consequences are virtually unexplored. This is despite the fact that Rawson (1939) recognized the fundamental importance of lake size as a factor in fish production more than 50 yr ago. Even less known, but of potentially greater ecological importance, are size-related transitions — and rapidity of transitions — between fundamentally different physical states (unfrozen to frozen, unstratified to stratified, dimictic to monomictic). Broecker (1987) pointed out that it is characteristic of natural systems to remain unchanged in the face of fairly broad ranges of a perturbation, but to suddenly “flip” to radically different states when critical thresholds are exceeded. There is no question that size plays an important role in this regard: small, deep ELA lakes (areas 5–30 ha, depths >10 m) are ice-covered for 6 mo, strongly stratified for nearly 4 mo, and do not fully mix in the spring if the period immediately after ice-out is warm and calm (Schindler 1971b) whereas the offshore zone of nearby Lake Superior remains unfrozen all year, weakly stratifies for only 2 mo in the summer, and always fully circulates prior to the establishment of summer stratification (Bennett 1978).

Information on how the structure and function of aquatic ecosystems are related to size will have universal application but is particularly needed so that controlled whole-lake manipulation experiments can be credibly extrapolated to larger lakes. Such experiments are logistically feasible only in small lakes (Johnson and Vallentyne 1971; Schindler 1988), while their most important applications are in developing management schemes for larger lakes. Up to now, these results have been extrapolated to larger lakes without considering the potential modifying effects of lake size (ignoring the fact that the ratio of the volume of ELA L.227, the site of early eutrophication experiments, to that of Lake Superior is similar to the ratio of a 300-mL BOD bottle to that of L.227). Further, the many long-

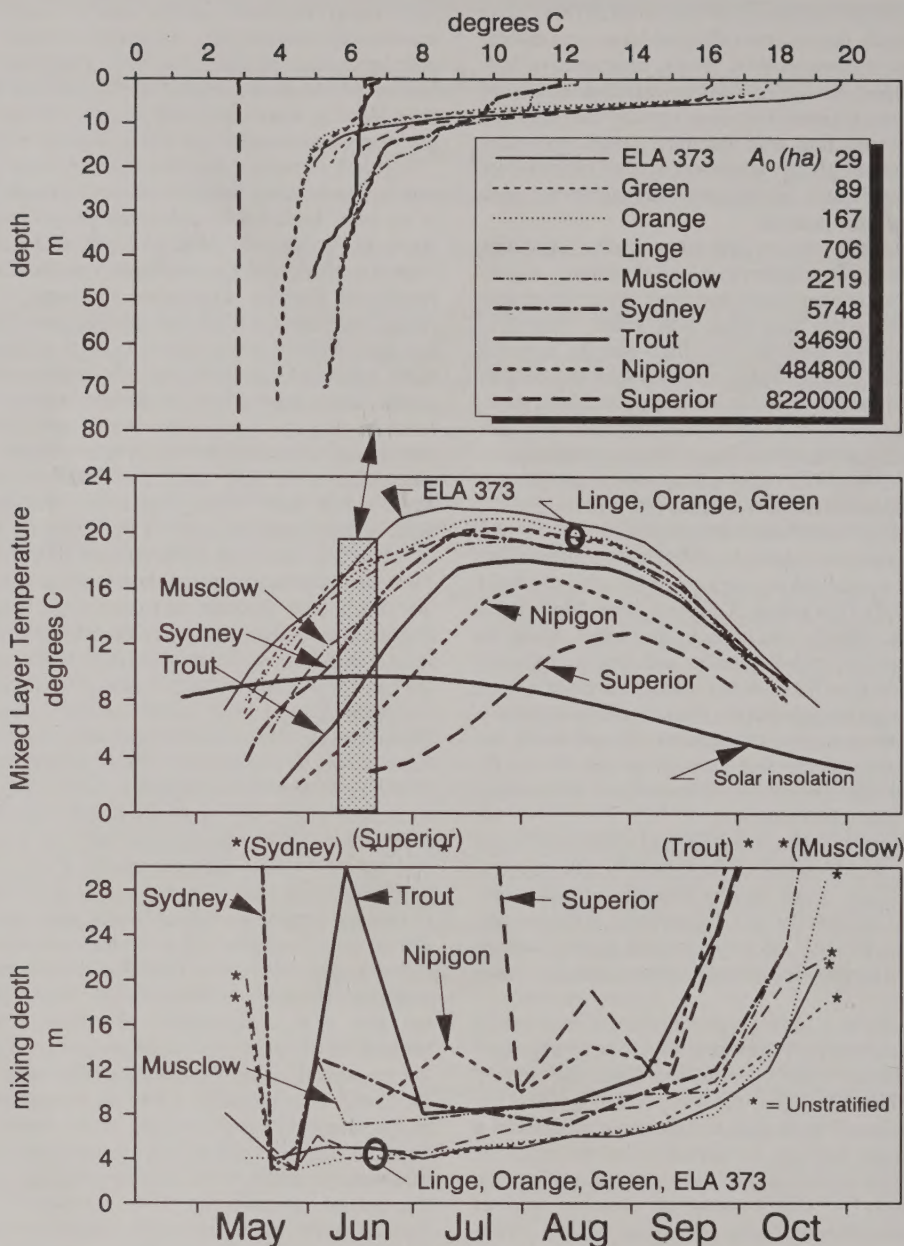


FIG. 1. Temperatures and mixing depths in a range of lake sizes in Northwest Ontario during 1990. Top panel: temperature as a function of depth during early summer (period shaded on the middle panel), the time when solar energy input is at its maximum and size-related temperature differences are greatest. Middle panel: mixed-layer temperatures from spring through autumn. Bottom panel: depth of the mixed layer from spring through autumn (asterisks indicate that the lake was unstratified).

term observations of unmanipulated lakes at ELA are now being analyzed for evidence of the initial effects of global warming (Schindler et al. 1990); information from a lake size series is needed so that relationships observed in these data can be rigorously applied to larger and more economically important lakes.

The Problem of Natural Variability

Meteorological variables that influence biological communities in lakes (precipitation, temperature, wind, and sunshine)

vary over all time scales. These variations cause natural variability — the broad range of natural states that occur in anthropogenically unperturbed lakes. Unambiguous identification of the specific effects of an anthropogenic perturbation is possible only if the perturbation effects are large relative to this natural variability, or if natural variability can be quantitatively modelled and thus removed from the record.

Ecologists have only recently recognized the fundamental importance of natural variability to their studies. "One wonders how many ecological 'responses' have been reported, which

are really just natural variation" (Schindler 1987); "Without an accurate knowledge of temporal complexity it is not possible to develop meaningful hypotheses" (Likens 1983). Examples from these papers illustrate the nature of the problem: changes of hydrogen ion and nitrate in the rain at Hubbard Brook (Likens 1983) and trends of transparency over time in an acid treated lake at ELA (Schindler 1987) — both originally interpreted as effects of acid rain — were shown by subsequent observation over longer time periods to be within the range of natural variability of unmanipulated ecosystems. On the other hand, pH trends in Swedish rivers (Likens 1983) and decadal to millennial changes in the abundance of marine fishes (Schindler 1987) demonstrate that significant but weak trends occur in both perturbed and unperturbed systems and can be detected by long time series, in spite of extreme short-term natural variability. The inescapable conclusion is that multiyear studies of ecosystems unaffected by point-source perturbations are required to appreciate and model the nature and magnitude of natural variability. Without this information, the interpretation of causality in perturbed systems is inherently ambiguous.

Greatly improved understanding of natural variability is particularly necessary for dealing with problems that cause slow, chronic (as opposed to dramatic) habitat degradation. As mentioned previously, the effects of acid rain demonstrate the importance of this type of chronic habitat degradation, and the issue of natural variability was prominent in the debate over the extent of damage caused by acid rain precipitation (Schindler 1989). Natural variability will be even more contentious in the future, since most global ecological problems will, like acid precipitation, act slowly and affect vast geographic regions over long periods. Monitoring programs are now being designed to detect these effects, but there is still debate over what lakes might be best suited for long-term monitoring because nothing is known about the factors that control the signal-to-noise ratio in different kinds of lakes.

Hypotheses

Likens (1983), Callahan (1984), and Schindler (1987) all agree that multiyear studies of natural ecosystems are needed to ensure wise and cost-effective management of natural resources. They decry the poor image of long-term monitoring studies, which have acquired an image of routinely measuring convenient rather than appropriate parameters. We believe that the dividing line between programs of pedantic, uninspired measurement and creative longterm research is the generation of specific hypotheses and the testing of these hypotheses with the acquired observations. It is appropriate, therefore, for this introductory paper to specify the hypotheses being tested in the NOLSS program:

(1) *Conclusions drawn from experimental manipulation and long-term monitoring of ELA lakes can be extrapolated in unmodified form to all sizes of Canadian Shield lakes in North-west Ontario.*

(2) *Natural interannual variability is an inverse function of lake size.* This hypothesis is extensively discussed in Fee et al. (1987).

(3) *Nutrients (i.e. nitrogen, phosphorus, and silicon) are the primary factors limiting algal photosynthesis and biomass in Canadian Shield lakes, regardless of size.* There are several reasons to suspect that nutrients may be less important as limiting factors in large lakes: it is known that nutrients, light, and temperature can colimit algal growth (Nalewajko et al. 1981; Healey 1985); as lakes increase in size, light or temperature

may replace nutrients as the primary limiting factor. Second, because large lakes are much more turbulent environments than small ones (Quay et al. 1980), nutrients should be retained longer in the mixed layer, and the upward flux of nutrients through the summer thermocline should be greater in large lakes; both of these processes will tend to alleviate phytoplankton nutrient deficiencies. Finally, the number of fish species increases exponentially as a function of lake size (Matuszek and Beggs 1988); according to the trophic cascade hypothesis (Carpenter et al. 1985), this could cause algal populations in large lakes to be controlled by "top-down" (grazing effects) rather than by "bottom-up" (nutrient) effects.

(4) *The effects of watershed inputs of nutrients and organic matter are less dominant in large lakes than in small ones.* Small lakes on the Canadian Shield are essentially holes in the forest. Consequently, direct inputs of terrestrial materials per unit of lake area are likely to be greater in small lakes than in large ones. It is known that sediments from small Shield lakes contain more organic material than those from large lakes (Brunskill et al. 1971), but it is unclear whether this is the result of different rates of in-lake productivity/respiration or higher inputs of refractory terrestrial materials. It is important to understand the processes that determine organic content of sediments because the composition of sediment — especially its organic content — controls the partitioning of nutrients and toxic chemicals between sediment and water (Eisenreich et al. 1989).

(5) *Community structural complexity and composition are functions of lake size.* Fish species/lake area curves are well documented (Matuszek and Beggs 1988), but it is not known whether this relationship applies to lower trophic levels (zooplankton, phytoplankton), whether it applies to the pelagic region (once "edge" effects are removed), or whether it applies within trophic levels (are the ratios of copepods to cladocerans, or of diatoms to cyanophytes, functions of lake size?).

(6) *Ecosystem productivity and trophic structure (e.g. phytoplankton photosynthesis, zooplankton production, fish growth) are independent of lake size.* An important corollary of this hypothesis is *Cycling of materials (including pollutants) within lakes is independent of size.*

NOLSS Experimental Design

NOLSS was designed to test the above hypotheses by accomplishing the following objectives:

(1) To determine how lake size (surface area) relates to biological productivity, community structure, and cycling of materials. In particular, to use a range of lake sizes to investigate the effects of temperature and length of the growing season on biological structure and function.

(2) To establish the applicability of experimental and long-term monitoring studies performed on small lakes to larger lakes (especially the Laurentian Great Lakes), and vice versa.

(3) To determine whether the magnitude and pattern of natural temporal variability of physical, chemical, and biological properties at time scales of seasons to years are functions of lake size.

(4) To document baseline conditions in lakes spanning a significant range of sizes in a remote, wilderness region. The purpose is to facilitate identification of future impacts of global changes.

Figure 2 shows the NOLSS design: six lakes that have similar watershed area/lake area ratios and that range in surface area from 89 to 34 700 ha (maximum overwater wind fetch 1–

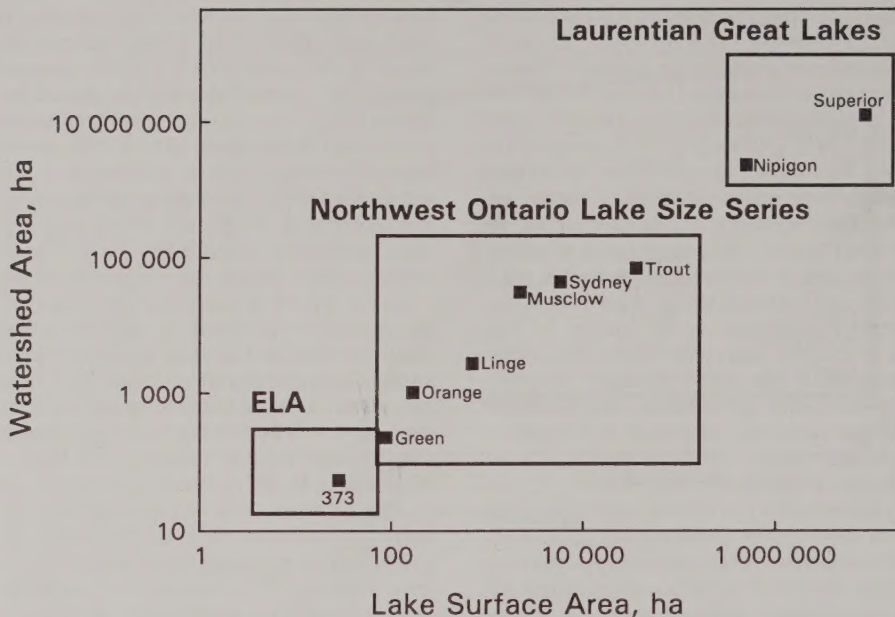


FIG. 2. NOLSS experimental design. The NOLSS lakes fill the gap that separates ELA lakes from the Laurentian Great Lakes.

28 km). The theoretical basis of this design is that because of their greater exposure to wind and the fact that the Coriolis force is insignificant in small lakes (Mortimer 1955; Boyce 1974; Patterson et al. 1984), large lakes are more energetic environments than small ones. This energy gradient systematically affects the four interfaces through which materials and energy pass to and from the mixed layer of stratified lakes: (1) the air/water interface, (2) the interface between the more buoyant epilimnion (mixed layer) and the hypolimnion, (3) the sediment/water interface, and (4) the terrestrial interface (i.e. the character of inflowing streams, shoreline characteristics). In turn, the changing character of these interfaces affects biological structure and function. NOLSS is designed to expose these environmental gradients and examine their biological effects.

The NOLSS lakes all have similar watershed area/lake area ratios; this ratio was chosen so that they would have water turnover times greater than 5 yr; they also share a common geology (Canadian Shield) and climate (cold temperate). Other types of lakes — unstratified, fast flushing, located in different geological settings, or in different climates — may be affected by size-related energy gradients in fundamentally different ways. The specific effects on natural temporal variability associated with significant variations of water renewal time (which affects the degree of linkage to terrestrial ecosystems) and lack of thermal stratification (which affects the intensity of internal nutrient recycling; Fee 1979) are currently being investigated by P. Campbell and co-workers, who are studying small lakes at ELA (surface lake ~30 ha, fetch <1 km) which is the only part of the size spectrum with enough lakes to permit systematic investigation of these effects.

The NOLSS lakes are located just east of the Manitoba/Ontario border in Red Lake District of Northwest Ontario (Fig. 3). This region (51°N, 94°W, 150 km north of ELA) was selected for its remoteness (air access only), its abundance of

deep lakes of different sizes, its overall similarity to ELA (Precambrian Shield geology, cold continental climate; see Brunskill and Schindler 1971 for details), and because it is conveniently sampled from either the Freshwater Institute in Winnipeg or ELA, which permits efficient monitoring by eliminating the need for a separate field laboratory. In the summer of 1985, 115 lakes in the Red Lake District were surveyed; a surface water sample was taken for chemical analysis, water transparency was determined with a Secchi disk, and temperature as a function of depth was measured (Fee et al. 1989). From this data set, six lakes that conformed to the NOLSS design criteria were selected. Table 1 presents some of the characteristics of these lakes as well as values for smaller (ELA) and larger (Great Lakes) lakes in Northwest Ontario. Bathymetric maps of the NOLSS lakes were presented by Fee et al. (1989).

To the best of our knowledge, the NOLSS lakes are not significantly affected by point-source anthropogenic impacts, and we are confident that this condition will persist for the planned lifetime of NOLSS (a decade). The lakes are only accessible by air, and there are no permanent residents in their drainage basins. The four smallest lakes are located in Woodland Caribou Wilderness Park, in which future developments will be closely controlled; the two largest lakes are outside the park but are not threatened with any planned developments. The only human activity that might affect these lakes is that the four largest ones are sport-fished; but the fact that they are only accessible by air limits the intensity and facilitates monitoring of this activity. Atmospheric loading of contaminants is low: concentrations of sulfate (a general indicator of inputs of atmospheric contaminants) vary between 20 and 30 $\mu\text{mol}\cdot\text{L}^{-1}$. We are therefore confident that what we observe in the NOLSS lakes will be responses to size and regional atmospheric inputs, and not to lake-specific anthropogenic perturbations.

Two types of variables are measured in NOLSS (Table 2). "Core" variables are measured in all lakes throughout the ice-



FIG. 3. Map of Northwest Ontario showing the location of the NOLSS lakes. Nipigon and Superior sampling stations are marked with a white cross on a black circle.

TABLE 1. Morphometric and chemical parameters for Northwest Ontario lakes that conform to the NOLSS design criteria (fully stratified in summer, water renewal times >5 yr, Canadian Shield geology). A_0 , lake surface area (net water area, not including the area of islands), ha; A_d , area of the terrestrial drainage basin (not including the lake area), ha; $(A_d + A_0)/V$, ratio of total watershed area to lake volume; τ_w , nominal water renewal time, calculated from lake volume, total watershed area, and maps of mean annual runoff, yr; z_m , maximum depth, m; $\bar{z}$, mean depth, m; z_r , relative depth, the ratio of maximum depth to mean surface diameter, %; SLD, shoreline development (including islands in shoreline length; not calculated for Nipigon and Superior because the disparity in map scales is too great); A_e/V_e , ratio of epilimnion sediment area to epilimnion volume in midsummer, m^{-1} ; k_{25} , specific conductance (at 25°C), $\mu S \cdot cm^{-1}$.

	A_0	A_d	$\frac{(A_d + A_0)}{V}$	τ_w	z_m	$\bar{z}$	z_r	SLD	A_e/V_e	k_{25}
ELA										
L.373	29	54	0.278	13.5	21	10.3	3.46	1.54	0.074	26
NOLSS										
Green	89	234	0.474	8.6	19	7.7	1.79	2.02	0.081	28
Orange	167	1 100	0.529	7.8	29	14.4	1.99	2.31	0.055	48
Linge	706	2 980	0.624	6.7	23	8.4	0.77	2.84	0.104	30
Musclow	2 220	32 850	0.821	5.0	46	19.2	0.87	3.64	0.032	43
Sydney	5 750	46 400	0.454	9.1	73	20.0	0.85	7.40	0.053	41
Trout	34 700	71 800	0.226	17.5	49	13.6	0.23	10.48	0.059	62
Great Lakes										
Nipigon	484 800	2 450 000	0.103	25.6	143	58.5	0.18		0.007	147
Superior	8 223 600	21 000 000	0.024	180.4	403	148.7	0.12		0.003	96

free season in all years; these results will provide information about both the mean effects of lake size and natural variability. Fee et al. (1989) gave extended descriptions of the methods used to measure the "core" variables, and we intend to adhere as closely as possible to these methods for the duration of the study. Methods are less rigidly defined, or frequency of measurement is less regular for "other" variables (Table 2), which are intended to elucidate only the mean effects of lake size.

Limitations of the NOLSS Design

NOLSS does not include the complete spectrum of lake sizes in Northwest Ontario (Fig. 2). Thus, to appreciate how size affects individual interfaces or processes in all lakes, information from lakes outside the NOLSS series would be required. Fortunately, a great deal of information already exists for both larger (Superior) and smaller (ELA) lakes of the region which

TABLE 2. Variables being measured in the NOLSS program. There are two classes of variables: (1) "core" variables, which are being monitored regularly and with unchanging methods (these will supply information needed for determining lake size effects and the magnitude of natural variability; no new variables will be added to this class) and (2) "other" variables, which are measured irregularly or with varying methods and will supply information relevant only to lake size effects (new variables will be added to this class as needed).

<i>"Core" variables</i>	
Standard meteorological variables (Ontario Ministry of Natural Resources meteorological station, Red Lake Ontario) and continuous records of solar insolation during the ice-free season	
Temperature as a function of depth	
Water transparency: Secchi disk depth and light attenuation as a function of depth (quantum meter)	
Phytoplankton photosynthesis versus light curves	
Physiological indicators of algal nutrient deficiency and nitrogen fixation rates	
Complete water chemistry	
In situ $p\text{CO}_2$ concentration	
Phytoplankton species composition and biomass	
Zooplankton species composition and biomass	
<i>"Other" variables</i>	
Microbial dark respiration rate ($p\text{CO}_2$ increase in the dark)	
Epilimnion and hypolimnion sediment respiration rates (O_2 uptake)	
Algal growth rate (^{14}C labelling of chlorophyll)	
Concentration of volatile sulfur compounds in the mixed layer	
Concentration of mercury in water and fish; rates of microbial methylation and demethylation	
Concentrations of airborne toxic chemicals (e.g. PCBs, furans, pesticides) in water, sediments, plankton, and fish	
Sedimentation rates and sediment chemistry	
Overwater wind direction and velocity	
Intensity of turbulent mixing between the mixed layer and the hypolimnion	
Concentrations of major and minor ions throughout the water column at the beginning and end of summer and winter	
Species composition and growth rate of fish	
Biomass of offshore fish species (dual-beam acoustic methods)	
Stable isotope composition of fish, water, and sediments	

are consistent with the NOLSS design (Canadian Shield geology, cold continental climate, long water renewal times).

More serious problems derive from the fact that no two lakes are exactly the same in every respect except size. Because Canadian Shield bedrock is all crystalline and Precambrian in age, it is widely considered to be a single geological entity. But the mineral content and physical structure of Shield bedrock as well as the nature and quantity of glacial debris on the Shield can vary tremendously (Stockwell et al. 1976). This results in individual differences of water chemistry and basin shape which have the potential to vitiate the NOLSS experimental design, which presumes that size is the only important variable in this group of lakes. Figure 4 shows how several important limnological characteristics vary among the NOLSS lakes and those identified above as logical extensions of the NOLSS design. The remainder of this section discusses the deviations from uniformity of the variables shown in this figure.

The most prominent deviations are at the large end of the size spectrum. This results, in part, from the fact that the option of excluding a lake because it deviates in some way from the others decreases sharply in the larger size categories. That is, while there are seemingly innumerable Shield lakes with areas of around 100 ha, few are greater than 10 000 ha, and there is only one Lake Superior. On the other hand, some parameters, for example mean depth (Koshinsky 1970) and A_e/V_e (Fee 1979), vary systematically with size and we do not consider the deviations shown in Fig. 4 to be complications of the NOLSS design. That is, large lakes having the same mean depth

or A_e/V_e as small ones would not satisfy a basic requirement of the NOLSS design because they would not stratify during the summer.

More worrisome are size-related deviations that are due to local geological inhomogeneities. Thus, Lakes Nipigon and Superior have the highest electrical conductivity (k_{25}) values (147 and 96 $\mu\text{S}\cdot\text{cm}^{-1}$, respectively), while the small ELA lakes have some of the lowest k_{25} values reported in the literature (11–30 $\mu\text{S}\cdot\text{cm}^{-1}$; Armstrong and Schindler 1971). This pattern results from the fact that the basins of Lakes Nipigon and Superior contain an abundance of both relatively soluble meta-volcanic–metasedimentary greenstone bedrock (Stockwell et al. 1976) and carbonate-containing glacial materials (at one time, these lakes were along the main outflow channels for glacial Lake Agassiz; Lemoine 1989). In contrast, the ELA bedrock is virtually insoluble granodiorite (Brunskill and Schindler 1971) and glacial deposits are sparse or absent because ELA sits at the top of a regional topographic high point and much of it was thus unaffected by glacial Lake Agassiz. If we assume that the probability of encountering soluble bedrock and/or glacial debris increases with both decreasing elevation and the size of the basin, then it is easy to explain why k_{25} values in the NOLSS lakes are intermediate between the ELA and Nipigon/Superior extremes.

While variations of k_{25} per se in the range shown in Fig. 4 are unlikely to be ecologically significant, some elements that vary in the same manner as k_{25} could be. For example, calcium ranges in concentration from 50 to 100 $\mu\text{mol}\cdot\text{L}^{-1}$ in ELA lakes

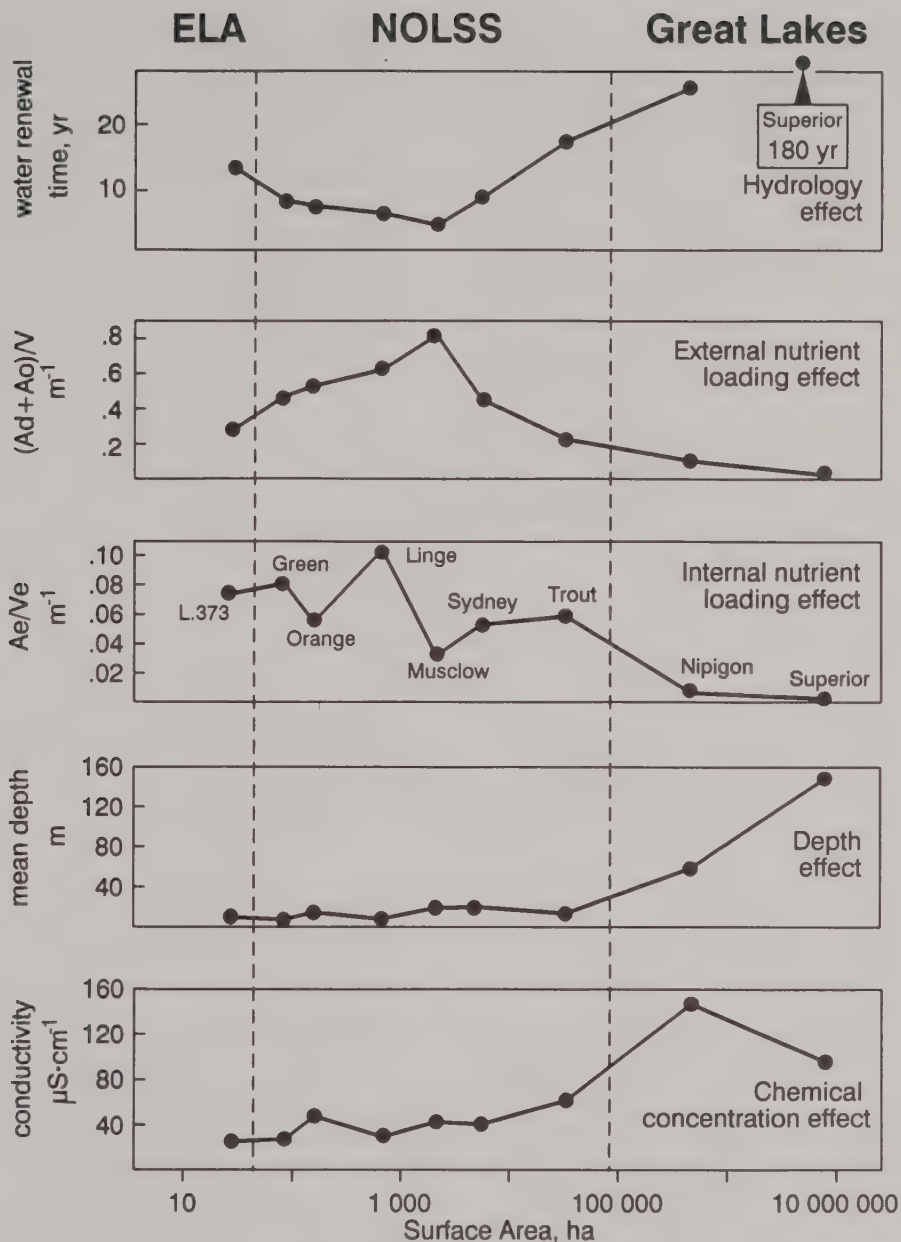


FIG. 4. Comparison of important features of the NOLSS lakes with ELA lakes and the Great Lakes (Nipigon, Superior).

to 600 and 350 $\mu\text{mol}\cdot\text{L}^{-1}$ in Nipigon and Superior, respectively. Some mollusc species are only found in waters where calcium $>250 \mu\text{mol}\cdot\text{L}^{-1}$ (D. Malley, pers. comm.), while the cladoceran *Holopedium gibberum* and some species of desmids and dinoflagellates are only found in waters where calcium $<250 \mu\text{mol}\cdot\text{L}^{-1}$ (Hutchinson 1967). Whether variations of calcium over the range seen in the NOLSS lakes (40–130 $\mu\text{mol}\cdot\text{L}^{-1}$) are ecologically significant remains to be determined.

Nutrients (phosphorus, nitrogen, and silicon) are chemicals that contribute essentially nothing to k_{25} but play a critical role in determining algal biomass and productivity. Because one of the main goals of NOLSS is to determine whether productivity

is a function of lake size per se, nonuniformity of the factors that control the availability of nutrients in the NOLSS lakes could complicate interpretation of NOLSS results. Three of the parameters in Fig. 4 have been shown to be important in this regard: (1) water renewal time (τ_w ; labelled "hydrology effect"), (2) input of nutrients from the watershed ($(A_d + A_0)/V$; labelled "external nutrient loading effect"), and (3) recycling of nutrients from epilimnetic lake sediments (A_e/V_e ; labelled "internal nutrient loading effect").

NOLSS was specifically designed to avoid complications associated with variations of τ_w . Vollenweider (1976) showed that when τ_w is less than 1 yr, nutrients can be "washed-out" of a lake before they are fully processed (i.e. taken up by algae

and lost to sedimentation). But when τ_w is longer than 5 yr — as it is in all the NOLSS lakes — its influence is negligible.

The situation regarding the other two parameters related to nutrient availability is less clear. If it can be assumed that rain is the only significant source of nutrients in Shield watersheds, and further assumed that over long time periods the watershed upstream of a lake exports the same amount of nutrients as it receives as input from the atmosphere (i.e. it is in “steady state”), then it follows (Schindler 1971a) that external nutrient inputs to a lake will be proportional to the total catchment area of the lake ($A_d + A_0$, where A_d is the area “upstream” of the lake and A_0 is the area of the lake) and external loading is thus proportional to $(A_d + A_0)/V$, where V is the volume of the lake. Under this hypothesis, external nutrient loading varies by nearly four times within the NOLSS lakes (Fig. 4).² However, the assumption that the watershed of a lake is in “steady state” with regard to nutrients is certainly incorrect if the watershed contains wetlands or long residence time lakes — which is invariably the case for large Shield lakes — because these ecosystems selectively retain nutrients (Schindler et al. 1973, 1976; Schindler and Fee 1974; Hecky unpubl.).

Fee (1979) showed that the rate of primary production (PP; phytoplankton photosynthesis) in small Shield lakes increases in proportion to the ratio of the area of epilimnion sediments, A_e , to the volume of the epilimnion, V_e . He deduced that this relationship is causal and reasoned that the rate of nutrient recycling in a lake is proportional to A_e/V_e . Figure 4 shows that A_e/V_e varies by threefold in the NOLSS lakes. However, there are reasons to expect A_e/V_e to be an inadequate parameterization of nutrient recycling in larger lakes. This expectation is based on the fact that the shape of the basin (A_e/V_e) is only one factor that could affect the rate of nutrient recycling; other obvious factors (e.g. the thickness of the mixed layer and the intensity of turbulence) that are either uniform or negligible in small lakes are unquestionably important in larger lakes (Quay et al. 1979, 1980; Gorham and Boyce 1989).

There is thus reason to suspect that the variations of $(A_d + A_0)/V$ and A_e/V_e shown in Fig. 4 will not be ecologically important. Moreover, the values of these ratios seen in the NOLSS lakes cover only a small part of the range seen in the ELA lakes, where they were shown to be statistically significant: Schindler's (1971a) $(A_d + A_0)/V$ values ranged from 0.1 to $4.2 \cdot \text{m}^{-1}$ compared with 0.1–0.8 in NOLSS lakes, and Fee's (1979) A_e/V_e values ranged from 0.01 to $0.66 \cdot \text{m}^{-1}$ compared with 0.03–0.10 in NOLSS lakes. If only the ELA data that fall within the narrower range of the NOLSS lakes are included, few of Schindler and Fee's regressions would remain significant.

Applications of the Results of NOLSS Studies

There are a number of practical reasons for studying a series of lakes which, while comparable in climatic, geological, and morphological characteristics, encompass a broad range of sizes:

(1) *To identify a lake size that would be a suitable “standard” for interregional comparison studies.* There may exist a unique lake size which is associated with inherently low tem-

poral variability (Fee et al. 1987). If so, then regional characteristics could be determined with the least sampling effort by studying lakes of this size in different regions. Models derived from studies of these lakes could subsequently be applied to individual lakes of different sizes in different regions with a minimum of site-specific work. This theoretical framework is needed for synthesizing the avalanche of short-term ecological observations being produced by site-specific environmental impact assessments.

(2) *To provide data needed for modelling the effects of climate change.* Current global atmospheric circulation models indicate that changes of climate associated with a doubling of atmospheric CO_2 will be severe in the NOLSS region (increases of $>9^\circ$ in mean summer temperatures and decreases of $>50\%$ in soil moisture content are predicted; Hengeveld 1990). Such changes would have profound effects on water quality and fisheries in the lakes of the NOLSS region, but our ability to predict what these effects might be is currently limited because existing long-term databases are available only for small ELA lakes (Schindler et al. 1990). As lakes increase in size, they become cooler and they remain ice-free for longer periods (Fig. 1). Therefore, a lake size gradient at one latitude provides gradients of important climatic variables that would be affected by global warming, and the response of biological communities to these gradients can provide information needed to model these impacts. In particular, the middle panel of Fig. 1 shows that, in the NOLSS lakes, differences of water temperature are cleanly separated from the seasonal distribution of radiant energy (which is the same for all these lakes). Because changes in the separation of seasonal warming from radiant energy input are precisely what global circulation models predict, lake size is arguably the best available real-time proxy for the effects of climate change on lakes.

An obvious alternative to the NOLSS approach is to compare data from “standard” lakes located in widely separated climatic zones. This was one of the goals of the International Biological Program (IBP; LeCren and Lowe-McConnell 1980; Brylinsky and Mann 1973), but a decade of IBP work resulted in very little information that is useful in modelling the effects of climate change because the lakes studied under IBP differed not only in their climatic settings, but also in their water renewal times, depths, geological settings, etc., intersite coordination was poor — different variables were measured at each site and different methods were used to measure the same variables, and individual studies were unfocused because data were not being gathered to test specific hypotheses (Schindler 1978; Fee 1979). In retrospect, the goals of IBP were laudable, and NOLSS has profited enormously from the IBP experience. Nevertheless, many of the problems that plagued IBP remain unresolved; for example, it is still not possible to define a “standard” lake in a meaningful way (this is a goal of NOLSS). Moreover, the probability that extreme weather events (e.g. years when it is unusually warm and dry versus years when it is cool and wet) will be synchronous decreases as the distance between study sites increases. Finally, in this approach, temperature and daylength both vary, while climate change will produce changes of only temperature and not daylength.

(3) *To fill the gap that exists between well-studied Canadian Shield lakes at the extremes of the size spectrum.* Figure 2 shows the enormous difference in size that separates ELA lakes (5–50 ha) from the Laurentian Great Lakes ($>1\,000\,000$ ha). Important physical features (date of ice-out, heating rate in the spring, maximum summer temperature and the time it occurs, depth of the mixed layer, thermal stability during the stratified

²The inverse relationship between $(A_d + A_0)/V$ and τ_w shown in Fig. 4 is not coincidental; it results from the fact that the assumptions made to calculate τ_w are the same as those made to calculate external nutrient loading, viz. rain is the only source of water (nutrients), terrestrial systems are in steady state with respect to water (nutrients), and the dilution volume is V .

period, and duration of the stratified period) vary tremendously over this size range (Fig. 1). Because of this, the applicability of existing information from the ends of the size spectrum to intermediate-sized lakes is fraught with uncertainty. Yet it is these intermediate-sized lakes that are the most heavily used for recreation because ELA-sized lakes are too small to support cottage development, while the Great Lakes are too cold or rough for most recreational uses.

(4) *To supply information needed for the interpretation of remotely sensed data.* Remote sensing technology can provide repetitive, unbiased, and standardized observations of important variables in all of the world's lakes, but accurate interpretation of such data requires site-specific information that is rarely available. For example, chlorophyll concentrations can be remotely sensed using the fluorescence line imager (Borstad et al. 1986), but estimation of phytoplankton productivity from chlorophyll data requires knowledge of biological transfer coefficients that have been reported to vary by more than an order of magnitude among different waterbodies (Platt and Subba Rao 1975); this makes remotely sensed chlorophyll data per se unsuitable for many purposes. However, if the mean values and variability of these transfer coefficients are consistent functions of lake size, or if interannual variability proves to be temporally synchronous over large regions, this technology could be the key to measuring biological productivity globally.

(5) *To provide baseline information.* Climate change, long-range atmospheric transport of toxic chemicals, and atmospheric ozone depletion could affect lakes in all parts of the world. It will be difficult to identify these impacts if we do not know the characteristics of unaffected lakes. Observations from a lake size series in a wilderness environment will provide this urgently needed information. Because such areas are increasingly rare and less remote, and because these problems are steadily increasing in scope and intensity, the window of opportunity for making baseline observations is rapidly closing. This is likely to be the most important long-term contribution of NOLSS.

Conclusion

NOLSS originally developed out of what was perceived as a limitation of the ELA experimental design, viz. that while only small lakes can be manipulated, the important application of these experiments is to lakes many orders of magnitude larger. In their original description of ELA, Johnson and Vallentyne (1971) recognized this as a potential problem, but ELA has operated for 20 yr under the unproved assumption that small-lake results could be applied to lakes of all sizes. There is no question that over this time, ELA has provided unique and important insights into how lakes function (Anonymous 1990). NOLSS will strengthen this experimental approach by providing knowledge of functional relationships required to rigorously extrapolate small-lake results to larger lakes.

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Effects of Lake Size on Phytoplankton Photosynthesis¹

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Phytoplankton photosynthesis (PP) was measured for 6 yr in seven remote Canadian Shield lakes that stratify fully during the summer and have water renewal times >5yr but vary from 29 to 34 700 ha; Lakes Nipigon and Superior were also studied in two years. Chlorophyll and PP at optimum light were low in the smallest and largest lakes and increased systematically to values nearly five times higher in midsized lakes (~10³ ha). Daily PP per square metre of lake surface and annual PP per cubic metre of the mixed layer also varied in this manner, but annual PP per square metre was high in large lakes (despite their low density rates) because of their long growing seasons. Additional data are needed to determine whether the photosynthesis maximum in midsized lakes is inherently size related or an accidental statistical result. Intraannually, chlorophyll-based photosynthesis parameters (P_m^B , α^B) were similar in all lake sizes, but interannually they varied by two to three times; this interannual variation was significantly correlated with total rainfall during May and June. Implications for extrapolating experimental results from small to large lakes, selecting lakes for interregional comparison studies, and predicting how climatic warming would affect phytoplankton photosynthesis are discussed.

Nous avons mesuré la photosynthèse du phytoplancton (PP) pendant 6 ans dans sept lacs éloignés situés dans le Bouclier canadien, qui se stratifient complètement au cours de l'été et présentent un taux de renouvellement de l'eau supérieur à 5 ans, mais dont la superficie varie de 29 à 34 700 ha. Les lacs Nipigon et Supérieur ont également été étudiés pendant deux années. Dans des conditions d'éclairement optimales, nous avons enregistré des valeurs faibles pour la chlorophylle et la PP dans les lacs de petites et de grandes dimensions, et des augmentations systématiques de ces facteurs jusqu'à des valeurs presque cinq fois plus élevées dans les lacs de dimensions moyennes (environ 10³ ha). La PP par mètre carré quotidienne de la surface des lacs et la PP par mètre cube annuelle de la couche de mélange des eaux variaient également de la même façon, mais la PP par mètre carré annuelle était élevée dans les grands lacs (malgré leur faible taux quotidien) en raison de la longue saison de croissance. Il faut recueillir des données additionnelles afin de déterminer si la photosynthèse maximale des lacs de dimensions moyennes a une relation systématique avec la dimension des lacs ou s'il s'agit d'un résultat statistique de nature accidentelle. Au cours d'une même année, les paramètres relatifs à la photosynthèse de la chlorophylle (P_m^B , α^B) étaient similaires pour les lacs de toutes les dimensions, mais d'une année à l'autre, ils variaient de l'ordre du double ou du triple; il y avait une corrélation étroite entre ces variations interannuelles et les précipitations totales enregistrées en mai et en juin. Enfin, nous abordons les répercussions de l'extrapolation des résultats d'expériences sur les lacs de petites dimensions à des lacs de grandes dimensions, dans la sélection des lacs aux fins d'études comparatives interrégionales, et sur les prévisions concernant les effets du réchauffement climatique sur la photosynthèse du phytoplancton.

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This paper is one in a series that address the question "What is the importance of a lake's size to its structure and function?" Fee and Hecky (1992) present a general introduction to these Northwest Ontario Lake Series (NOLSS) studies and present several hypotheses that NOLSS is designed to test. Prominent among these is the null hypothesis that ecosystem productivity is unrelated to lake size (hereafter referred to as the *Size → Productivity* null hypothesis). All ecosystem productivity depends ultimately on the fixation of solar energy by plants, i.e. photosynthesis, which is thus referred to as primary production (Lindeman 1942), and Schindler et al. (1973) showed that the only quantitatively significant photosynthetic process in deep, slowly flushed Canadian Shield lakes is phytoplankton photosynthesis (PP).² Thus, a logical way to test the

Size → Productivity null hypothesis is to determine whether PP varies in a systematic and logical manner in a series of lakes which are as similar as possible in every regard except size. That is the primary purpose of this paper.

Study Sites

Table 1 summarizes some limnological features of the studied lakes; more complete descriptions are given in Fee and Hecky (1992). These lakes all share a common geology (Canadian Shield) and climate (cold temperate); they are deep enough to fully stratify during the summer, and they have water renewal times longer than 5 yr. Except for Nipigon and Superior, there are no permanent residents in the drainage basins of these lakes, they are not significantly affected by point-source anthropogenic contamination, and recreational usage is limited to fly-in sport-fishing. Only Lakes Nipigon and Superior are commercially fished.

Although the basin of Lake Superior contains several permanent settlements (the largest being Duluth-Superior in the

¹This is one in a series of papers describing work of the Freshwater Institute Science Laboratory on a lake size series in Northwest Ontario.

²This is frequently not true in very small, shallow, or rapidly flushed lakes, and the term "primary production" should not be used synonymously with phytoplankton photosynthesis (Flynn 1988).

TABLE 1. Summary of major features of the studied lakes. A_0 , lake surface area (net water area, i.e. not including the area of islands), ha; A_d , area of the terrestrial drainage basin (i.e. not including the lake area), ha; A_d/A_0 , the ratio of terrestrial watershed area to lake surface area; A_e/V_e , midsummer ratio of epilimnion sediment area to epilimnion volume, m^{-1} ; lake volume, m^3 ; $\bar{z}$, mean depth, m; τ_w , nominal water renewal time, calculated from lake volume, basin area, and maps of mean annual runoff, yr; k_{25} , specific conductance (at 25°C), $\mu S \cdot cm^{-1}$. Note: bathymetric maps do not exist for Lake Nipigon; the underlined values in the table were calculated by assuming that Nipigon's area versus depth curve has the same shape as that of Superior.

Lake	A_0	A_d	A_d/A_0	A_e/V_e	Volume	$\bar{z}$	τ_w	k_{25}
ELA L.373	29	54	1.86	0.074	2.984×10^6	10.29	13.5	26
Green	89	230	2.64	0.081	6.820×10^6	7.68	8.6	28
Orange	167	1 100	6.60	0.055	2.400×10^7	14.37	7.8	48
Linge	706	2 980	4.22	0.104	5.910×10^7	8.37	6.7	30
Musclow	2 220	32 850	14.80	0.032	4.270×10^8	19.23	5.0	43
Sydney	5 750	46 400	8.08	0.053	1.150×10^9	20.00	9.1	41
Trout	34 700	71 800	2.07	0.059	4.720×10^9	13.60	17.5	62
Nipigon	484 800	2 450 000	5.05	0.007	2.836×10^{11}	58.50	<u>25.6</u>	147
Superior	8 223 600	21 000 000	2.55	0.003	1.223×10^{13}	148.72	180.4	96

United States and Thunder Bay in Canada) and supports diverse resource-based industrial activities (mining, paper-making, and shipping being the most prominent), the scale of these perturbations is small relative to the volume of the lake, and it is generally considered to be in virtually pristine condition (Matheson and Munawar 1978). This is unfortunately not the case for Lake Nipigon, which superficially appears to be even more remote than Superior. Beginning in the 1920s, Nipigon and its basin were subjected to intensive commercial fishing, logging, and log driving. During the 1930s, a gold mine dumped wastes into the lake, and artificial regulation of water levels to support the water needs of downstream hydroelectric stations was begun. Development of the hydroelectric potential of the lake accelerated in the 1940s, when the Ogoki River was diverted into Nipigon. This diversion increased the basin area/lake area ratio from 5.0 to 7.8 and caused massive erosion along the diversion channel ($10^6 m^3$ of sediments was added to the lake between 1943 and 1972; Day et al. 1982). Most of this material has accumulated in Ombabika Bay (a large, turbid, shallow bay that connects to the main body of the lake by a narrow channel) and probably stimulated production in this bay, which now produces much higher fish yields than other parts of Nipigon (R. Salmon, Lake Nipigon Fisheries Assessment Unit, pers. comm.). But the effects of these perturbations on the open waters of this lake are unknown, and this lake was included in this study because it is the only deep, slow-flushed lake in Northwest Ontario that is intermediate in size between the largest NOLSS lake (Trout) and Lake Superior.

Methods

Daily and annual PP and daily mean light (more precisely defined as photosynthetically available radiation (PAR) — the total number of quanta between 400 and 700 nm) were calculated using the computer programs of Fee (1990). In brief, these programs use linear interpolation to integrate separate measurements of (1) the relationship between photosynthesis and light (the so-called “ P versus I ” curve, shown in Fig. 1), (2) the relationship between PAR and depth, (3) the relationship between PAR at the surface of the lake and time, and (4) the depth of the mixed layer (defined as the first significant break — usually $> 1^\circ C \cdot m^{-1}$ — in the in situ temperature versus depth profile).

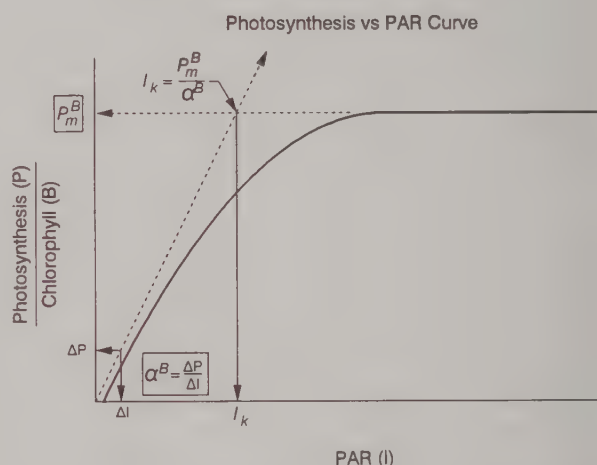


FIG. 1. Relationship between photosynthesis and PAR (defined in text). The parameters of this curve are as follows: P_m^B , the maximum; α^B , the slope of the light-limited part of the curve; I_k (P_m^B/α^B), the PAR below which photosynthesis is primarily light limited. The superscript B indicates that P_m^B and α^B are normalized on the biomass of photosynthetic pigments (chlorophyll a) in the water sample. P_{opt} (not illustrated) is the product of P_m^B and chlorophyll concentration; it is the unnormalized rate of photosynthesis at PAR optimal for photosynthesis.

Radiation and Temperature Measurements

PAR in the incubator used to measure P versus I curves was measured with a BioSpherical QSP 200 (spherical collector) quantum sensor. In situ attenuation of PAR as a function of depth was measured with a Li-Cor LI-185 underwater quantum sensor (flat plate, cosine-corrected collector).

All photosynthesis estimates reported here are based on actual (measured) solar values (i.e. not simulated cloudless values). These were measured with a Li-Cor LI-190SR quantum cell (flat plate, cosine-corrected collector) mounted at the top of a high tower (at Experimental Lakes Area (ELA) or a tall building (at Red Lake) to avoid shading. Simultaneous measurements were made at ELA and at Red Lake, Ontario, and as there were only minor differences in these records, data from either site were used to fill in gaps in records from the other site if a recording device failed. For the very rare periods when data

were not available from either site, it was assumed that 70% of cloud-free irradiance was received; this is the long-term mean measured at ELA (J. Shearer, unpubl.). ELA solar data were used to calculate *in situ* photosynthesis only in ELA lakes; the Red Lake solar data were used for all other lakes, including Nipigon and Superior.

Daily mean PAR in the mixed layer ($\bar{I}$) was calculated as

$$(1) \quad \bar{I} = \bar{I}_s \frac{\int_0^{z_m} A(z) I(z) dz}{\int_0^{z_m} A(z) dz}$$

where $\bar{I}_s$ is the mean solar flux at the surface of the lake during the day (24-h average), z_m is the mixing depth, $I(z)$ is the fraction of the solar PAR that penetrates to depth z , and $A(z)$ is the area of the lake at depth z . $\bar{I}$ was calculated twice: once with actual (measured) solar irradiance data and again with simulated cloud-free solar data (Fee 1990); the former is the best estimate of actual $\bar{I}$ levels, while the latter is better suited for elucidating trends over time, since day-to-day variations caused by cloud cover are removed.

Manual resistance thermometers were used to measure temperature as a function of depth in the NOLSS lakes in 1986 and 1987 and in all ELA lakes in all years. In other lakes and years, a free-falling temperature profiler that measured temperature at depth intervals of ~ 0.05 m was used (R. Brancker Research, Ltd., Ottawa, Ont.).

Solar irradiance was measured continuously and input to the computer programs as averages over 0.5-h intervals. The other light measurements were made throughout the ice-free season at intervals that varied between 1 and 3 wk (see below).

Field Sampling

Complete details of field and laboratory procedures and sampling locations are given in Fee et al. (1989). The six NOLSS lakes were sampled from the pontoon of a fixed-wing aircraft (de Havilland Beaver) anchored as close as possible to the position of maximum depth. ELA L.373 was sampled from a 3.7 m dinghy, and Lakes Nipigon and Superior were sampled with a 6.7-m Gimli yawl. In 1990, three deepwater stations were sampled on Nipigon (off Harbour Island, between Red Willow and Katatota Islands, and off 8-Mile Island); because data from all three of these sites varied in the same way over time, measurements were made at only the 8-Mile Island station in 1991, and only data from this station for 1990 are presented here. Our sampling station on Lake Superior was 5 km southeast of the east end of Isle Royale. In 1973, a large number of stations on Superior were regularly sampled from spring through fall, and the lake was subdivided into homogeneous regions; our site was included in the offshore zone with respect to temperature (Bennett 1978), transparency (Schertzer et al. 1978), and chlorophyll (Munawar and Munawar 1978). Our 1990 results confirm this classification, as they are virtually indistinguishable from values given as typical of the offshore zone in 1973.

In ELA lakes, water samples were integrated from the surface down to the first significant break in the temperature versus depth profile; in L. 373, this was usually 0–4 m but occasionally 0–5 m. In all other lakes, samples were integrated over a fixed depth range (0–3 m) which always included the top of the mixed layer. The sampler used was similar in principle to that described by Fee (1976), lacking only the apparatus needed for opening it at depths below the surface; all tubing on the sampler was composed of silicone, and all metal parts were coated with epoxy resin. In order to prevent exposure to bright light, the clear polycarbonate sample bottles were enclosed in

opaque PVC plastic casings. Samples were stored in insulated containers until arrival in the laboratory; holding times ranged from 2 to 6 h.

Because we sampled only the mixed layer, our results do not include PP in the metalimnion or hypolimnion. Studies at ELA showed that PP in these layers is $\sim 20\%$ of total PP in small Shield lakes (Fee et al. 1982) and that it is a function of the amount of light they receive (Fee 1978; Shearer et al. 1987). This percentage undoubtedly declines with increasing lake size because the increasing mixed-layer depth (Fee and Hecky 1992; and see Fig. 3) permits less light to reach these layers. Further, in ELA lakes, these layers have high rates of PP only when there are enormous concentrations of algal biomass in and below the thermocline (Fee 1976); such biomasses are never seen in these layers in larger lakes, presumably because they are more turbulent (Quay et al. 1980).

Samples were taken regularly throughout the ice-free season, starting soon after ice-out and concluding in mid-October. ELA and NOLSS lakes were sampled from 1986 through 1991; Lakes Nipigon and Superior were sampled in 1990 and 1991. ELA lakes were sampled starting in the week following ice-out (usually the second week of May) and samples were taken at intervals varying from 2 wk to 1 mo. L.373 (the lake that best matches the NOLSS design in regard to depth and water renewal time) was sampled at 2-wk intervals, except in the autumn, when samples were taken a 3-wk intervals. The NOLSS lakes were sampled at 3-wk intervals starting in mid-May in 1986–88; in 1989–91, they were sampled weekly from mid-May until the first week of June, the next sample was taken 2 wk later, and after that, samples were taken at 3 wk intervals. Lakes Nipigon and Superior were sampled at 3-wk intervals starting in mid-June. Because sampling during May was inconsistent from lake to lake and year to year, and because the entrainment of metalimnetic or hypolimnetic chlorophyll peaks can produce anomalous PP values in the fall (Fee 1976), PP totals are given both for the entire year and for the summer stratified months (June–August); the latter are the most reliable values to use for comparative purposes.

Laboratory Procedures

When the water samples were returned to the laboratory, they were subdivided for chemical analyses, plankton identification and counting, and photosynthetic rate measurements. Unless otherwise specified, chemical analyses were done using the methods of Stainton et al. (1977). Chlorophyll was determined by filtering 100 mL of water onto a GF/C filter; the filter was placed in a glass vial, 10 mL of 95% methanol was added, and the vial was placed in a freezer at -10°C and allowed to stand for 16 h; the extract was agitated, allowed to settle, and its fluorescence was assayed on a Turner model 110; the fluorometer was routinely standardized with a chlorophyll solution (Sigma Chemicals) whose stability was routinely verified with an HP scanning spectrophotometer. In 1986, chlorophyll extracts were not always mixed prior to reading fluorescence; this introduced a serious and inconsistent bias, and data from this year are therefore not reported. Although the same method was used throughout, ELA chlorophyll samples were processed in a different laboratory than those from the other lakes.

Fresh whole-water samples were inoculated with trace amounts of $\text{NaH}^{14}\text{CO}_3$, placed in 60-mL Pyrex bottles, and exposed for 3 h to a range of invariant light levels (five at ELA, 10 in the other lakes). Incubation temperatures were within 2°C of the *in situ* temperatures, and all laboratory materials were nonmetallic. ^{14}C uptake was measured in both the light and dark;

uptake in the dark was subtracted from that in the light. Detailed descriptions of radiochemical methodology and calculation formulae are given in Shearer et al. (1985). Our methods deviated from those described by Shearer et al. (1985) in two ways (see Fee et al. 1989 for details): (1) Instead of dispensing lake water into the 60-mL bottles and then inoculating these individually with ^{14}C , we added ^{14}C to the whole sample, mixed this manually, and siphoned it (silicone rubber tube) into the bottles; the new method reduced variance by ensuring that all bottles have the same specific activity. (2) Instead of the rotating cylindrical incubator (metal halide lamp) described by Shearer et al. (1985) which we used at ELA, a nonrotating rectangular incubator (high-pressure sodium lamp) was used for the NOLSS lakes and Nipigon and Superior. The new incubator is more compact, generates less heat, and exposes a sample at 10 instead of five light levels, thus probably giving more precise estimates of P_m^B and α^B . However, no statistically significant difference in mean parameter values was observed when a single sample was exposed simultaneously in the two incubators (during a 6-yr period, 64 samples from many ELA lakes were analyzed in this way; the following regression lines were highly significant, with slopes not significantly different from 1.0:

$$P_m^B(\text{NOLSS}) = 1.01 \times P_m^B(\text{ELA}) - 0.14;$$

$$\alpha^B(\text{NOLSS}) = 0.85 \times \alpha^B(\text{ELA}) + 0.27).$$

Data Analysis

Photosynthesis parameters (Fig. 1) were estimated by the computer program PSPARMS (Fee 1990), which takes as input the chlorophyll concentration and a corresponding table of P versus I data; it iteratively fits (least squares) the following equation to these data:

$$(2) \quad P = \begin{cases} 0 & \text{if } I < I_k/20 \\ B P_m^B & \text{if } I \geq 2 I_k \\ B \alpha^B I' \left[1 - \frac{I'}{4 I_k} \right] & \text{otherwise} \end{cases}$$

where P is the rate of photosynthesis per unit volume of water, B is the chlorophyll concentration, I is PAR, $I_k = P_m^B/\alpha^B$, and $I' = I - I_k/20$. As output, PSPARMS estimates P_{opt} (the rate of photosynthesis at light levels optimum for photosynthesis), P_m^B (the light-saturated rate of photosynthesis per unit of chlorophyll), and α^B (the slope of the photosynthesis versus light curve as light approaches zero per unit of chlorophyll). P_{opt} is the product of P_m^B and the chlorophyll concentration.

In situ photosynthesis and daily mean light in the mixed layer were estimated by the programs YPHOTO and YTOTAL, respectively (Fee 1990). Input to these programs consists of seasonal variations of in situ light extinction, variation of surface solar irradiance at 0.5-h intervals for the entire ice-free season, seasonal variations of the photosynthesis parameters P_m^B and α^B , and seasonal variations of chlorophyll concentration. Photosynthesis as a function of light (depth) is simulated using equation 2, which explicitly ignores photoinhibition (Bower et al. 1987). All in situ photosynthesis rates were corrected for the diminution of lake contour area with depth (Fee 1980).

Results

Physical Conditions

Weather was extremely variable during this study (Fig. 2). Variation was greatest early in the ice-free season (April–June);

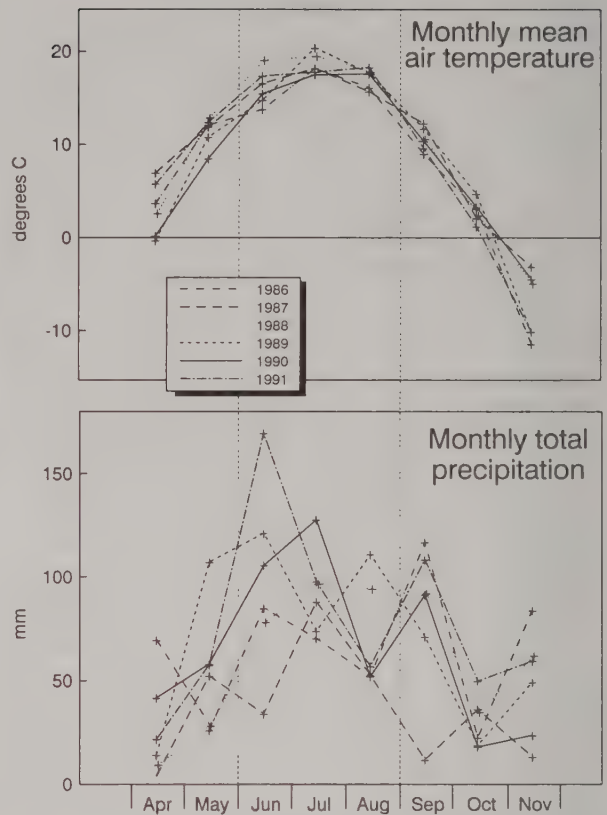


FIG. 2. Monthly mean air temperature and total precipitation at Red Lake, Ontario, during the ice-free seasons of 1986–91. The period June 1 to August 31 (the well-stratified summer period) is enclosed in broken vertical lines.

during these months the years 1987–88 and 1991 were $\sim 5^\circ$ warmer than the years 1986 and 1989–90, and in 1989–91, more than twice as much rain fell than in 1986–88.

In any given year, water temperatures were $\sim 10^\circ$ warmer in the smallest lake than in the largest lake, and the depth of the mixed layer was ~ 10 m shallower in the smallest lake than in the largest lake (Fig. 3). Interannually, water temperatures ranged $\sim 3^\circ$ in all lake sizes. Interannual differences in mixed-layer depths were much more erratic. This is probably due to inadequate temporal resolution of our mixing-depth data, i.e. samples taken every 3 wk cannot resolve — and thus properly average — phenomena that affect the depth of the thermocline (e.g. internal waves, upwelling). The notably greater variability of mixing depths in lakes ~ 1000 ha and larger is due to the fact that these phenomena only become significant in lakes greater than that size (Boyce 1974).

Components of the Photosynthetic System

Values of chlorophyll and P_{opt} were greatest in intermediate-sized lakes (Linge, Musclow) and, excepting Lake Nipigon, decreased in a regular fashion towards the extremes of the lake-size spectrum (Fig. 4). Maximum chlorophyll concentrations usually occurred immediately after ice-out in the spring, and minimum values usually occurred in early or midsummer, except in Lake Superior, which showed little seasonality. P_{opt} changed in a manner similar to chlorophyll but the peak in the spring was much less pronounced.

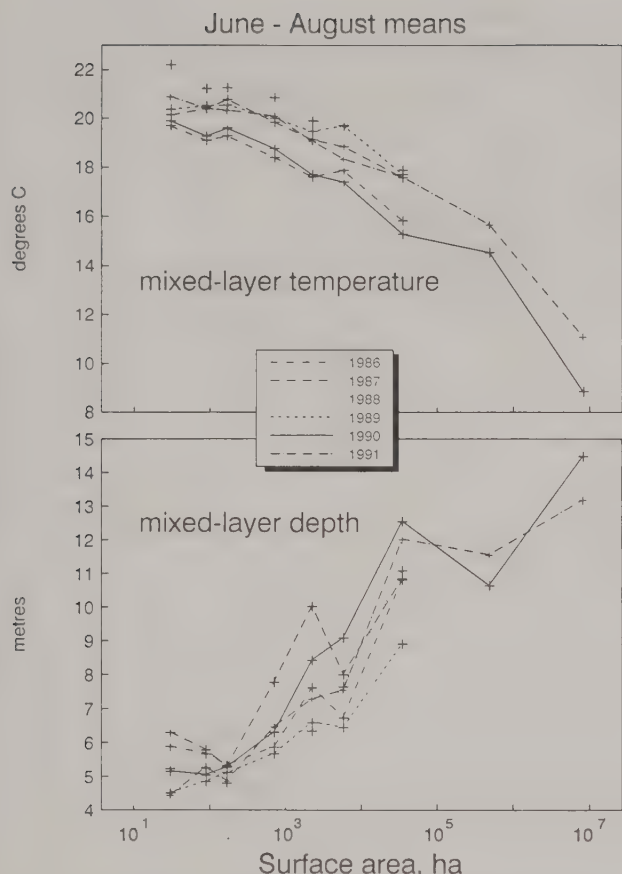


FIG. 3. Mixed-layer average temperature and depth during the period June 1 to August 31 in deep (well-stratified during the summer), slow-flushing (water renewal time >5 yr) Canadian Shield lakes in North-west Ontario.

In contrast with chlorophyll and P_{opt} , the photosynthesis parameters P_m^B and α^B (Fig. 1) showed no trend with lake size (Fig. 4). P_m^B values were usually low in the spring, high in mid-summer, and low again in the fall, while α^B values were low in the spring and then increased continuously until ice-up in the fall. This different seasonal behaviour suggests that the statistically significant relationship between P_m^B and α^B reported by many studies (and which is present in our data) is an artifact resulting from their normalization on a common variable (chlorophyll). While variations of P_m^B and α^B generally follow temperature and light, respectively (Jassby and Platt 1976; Hecky and Guildford 1984), these relationships are not strong (Fig. 5). Rather, as reported by Fee et al. (1987), our data show that temperature and light only set upper bounds on the variance of these parameters.

Because chlorophyll, P_{opt} , P_m^B , and α^B varied so much on individual sampling dates, it is difficult to appreciate differences in mean behaviour from the data just presented. In order to illustrate these differences, the mean values during the stratified period (June 1 to August 31) are shown in Fig. 6. Means of all variables ranged by two to three times interannually; in the cases of P_m^B and α^B , these changes were regionally synchronous except in the smallest (ELA) lake (Fig. 7). There was a significant correlation between summer mean values of P_m^B and α^B (but not of chlorophyll or P_{opt}) and total precipitation during May and June in the eight lakes larger than ELA L.373

(Fig. 8). Because rainfall is the main source of nutrients to the mixed layer of Shield lakes (Schindler et al. 1973), this indicates that these photosynthesis parameters are sensitive to changes in nutrient loading.

Figure 9 shows variations of daily mean PAR in the mixed layer ($\bar{I}$). $\bar{I}$ drops rapidly with increasing lake size in the two smallest lakes, but varies inconsistently as a function of lake size in lakes >100 ha.

In situ Photosynthesis Rates

Daily areal photosynthesis in the mixed layer (Fig. 10) varied in the same way as chlorophyll and P_{opt} , i.e. values were highest in intermediate-sized lakes and declined regularly (except in Nipigon) in both larger and smaller lakes. Summer and ice-free season integral rates (Fig. 11) deviated from this pattern in two ways. (1) Maximum areal rates (i.e. per square metre of lake surface) were in Musclove Lake, but maximum volumetric rates (i.e. per cubic metre of the mixed layer) were in Linge Lake. This results from the correction of PP depth integrals for the diminution of lake area with depth (Fee 1980), i.e. area decreases much more rapidly with depth in Linge Lake than in Musclove Lake (Fee et al. 1989). Uncorrected photosynthesis versus depth profiles (Fig. 12) clearly show that Musclove is more productive than Linge on a volumetric basis. (2) Seasonally integrated areal rates in lakes larger than Musclove do not decline consistently, even though volumetric rates do. This is largely a consequence of the sharp increase in mixed-layer depth in lakes larger than Musclove (Fig. 3). That is, in large lakes, PP is integrated over a greater depth range than in small lakes, which compensates for their declining volumetric rates (Fig. 12). The fact that larger lakes also have more ice-free days also causes their ice-free season totals to be high on an areal basis, but not on a volumetric basis because most of the water column is very poorly lit late in the growing season. PP was highest in years when precipitation during May and June was high (1989–91), indicating that nutrient loading limits PP.

Discussion

Size $\rightarrow$ Productivity Null Hypothesis

P_{opt} , chlorophyll concentration, daily areal PP, and PP per cubic metre of the mixed layer were all low and similar in magnitude in the smallest and largest lakes (L.373 and Superior) and, except for the one lake that has been grossly perturbed (Nipigon), increased systematically to maxima in intermediate-sized lakes (Fig. 4, 10, 11). This pattern is either a systematic feature of lake size beautifully exposed by the experimental design of this study or a serendipitous statistical accident resulting from flaws in this design.

Systematic lake size interpretation

Figure 13 shows a simple model in which two factors that control productivity change in opposite ways as lake size increases, resulting in optimum conditions in intermediate-sized lakes. The intensity of turbulence clearly increases with lake size (Quay et al. 1980; Ward 1977), and turbulence should stimulate phytoplankton photosynthesis because it makes nutrients more available to phytoplankton. It does this in several different ways. Vertical mixing following ice-out in the spring is a critical event in the annual cycle of a lake because it determines the initial amount of nutrients in the mixed-layer at the start of the stratified period (Goldman et al. 1989). Larger (more turbulent) lakes typically mix for prolonged periods at

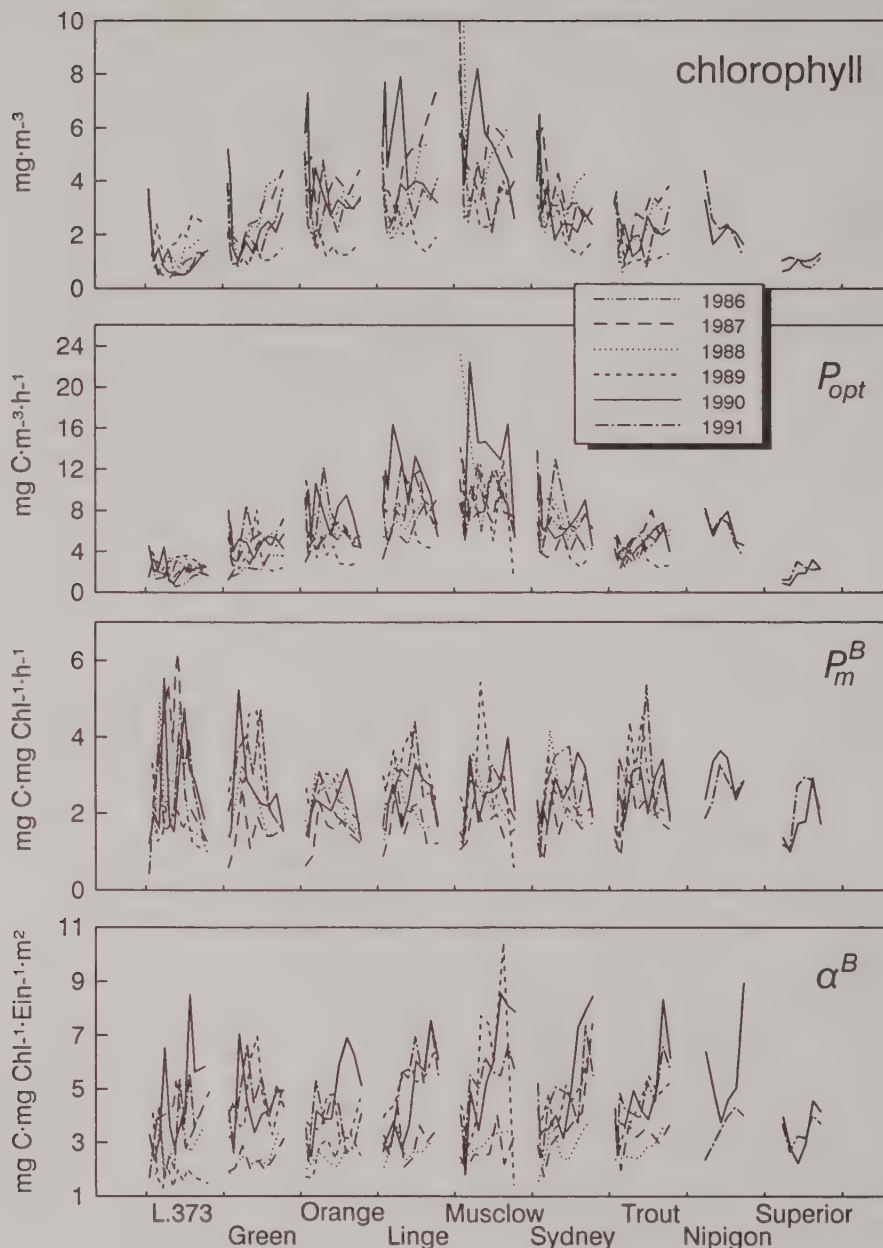


FIG. 4. Variations of chlorophyll, P_{opt} , P_m^B , and α^B during the ice-free season in lakes varying in size from 29 ha (ELA L.373) to 8 223 600 ha (Superior). The marks on the horizontal axis correspond to May 1 and December 1.

this time (but not always completely if they are very deep), while smaller lakes circulate for shorter periods — indeed, frequently not at all (Schindler 1971). More turbulent lakes also retain particles longer in their mixed layers (Margalef 1974), which leads to more efficient nutrient recycling. The flux of nutrients to the mixed layer from the hypolimnion during summer stratification depends directly on turbulence (Quay et al. 1980). Finally, turbulence enhances nutrient uptake by algae by reducing the thickness of the nutrient-depleted boundary layer surrounding algal cells (Hutchinson 1967; Margalef 1974).

It is more difficult to identify what factor(s) may cause PP to systematically decrease as lake size increases ("factor 2" in Fig. 13). Sterner (1990) reasoned that the increasing depth of the mixed layer with increasing lake size should cause $\bar{I}$ in the mixed layer to decrease and suggested that decreasing light availability could limit productivity in large lakes. Our results (Fig. 9) do not support this hypothesis. Except in the fall, $\bar{I}$ was always greater than values that typically limit PP. Further, although the ratio $\bar{I}/\bar{I}_k$ was <1 (implying light limitation) in all lakes >100 ha, there was no tendency for it to decrease progressively with increasing lake size. Finally, $\bar{I}$ was lowest in

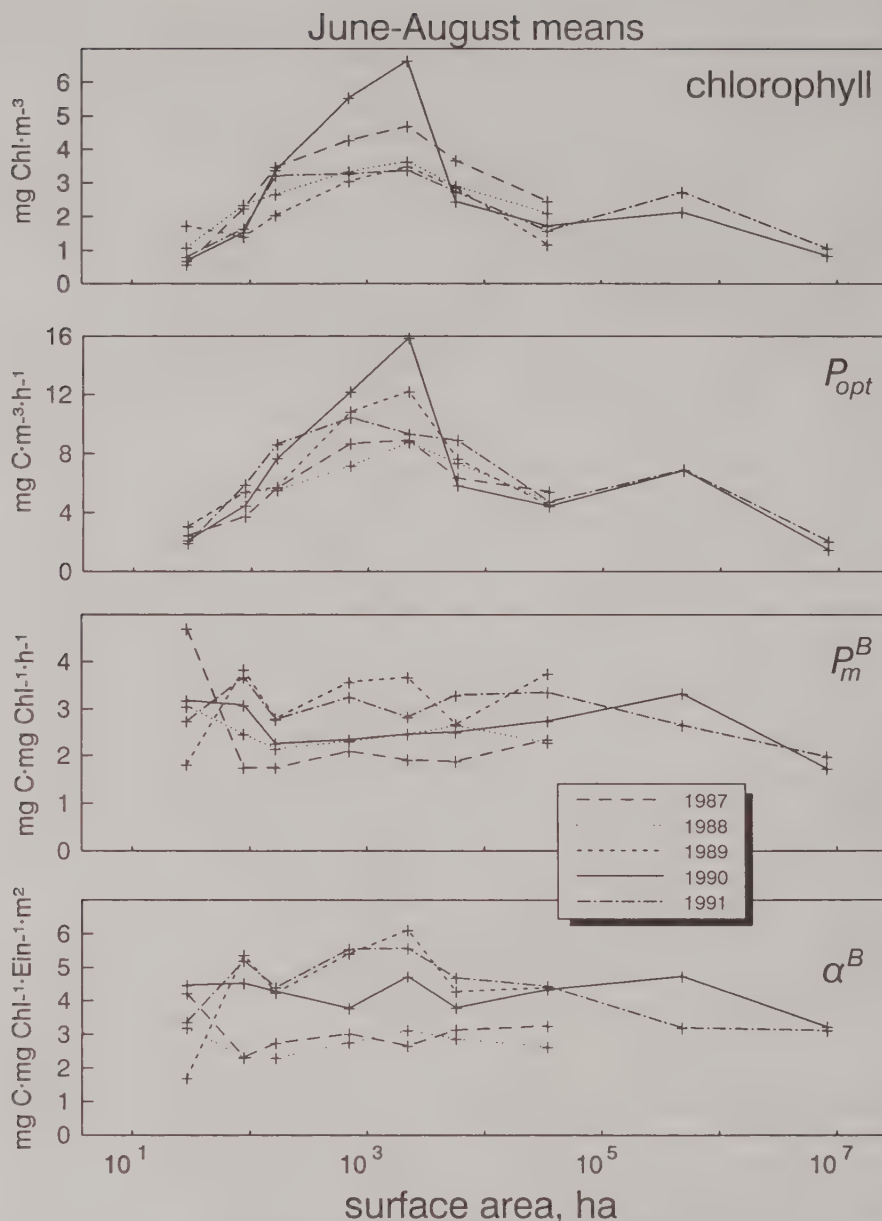


FIG. 6. Means for the stratified period (June 1 to August 31) of chlorophyll concentration, P_{opt} , P_m^B , and α^B plotted as a function of lake size.

Contradictory evidence to this interpretation is shown in Fig. 16, which compares chlorophyll and P_{opt} values from the most productive mid-sized lake (Muscow) with 12 small (5–56 ha) unmanipulated ELA lakes that match the design criteria for this study (deep enough to fully stratify in the summer, water renewal times >5 yr). In these small lakes the range of A_d/A_0 and A_e/V_e values exceeds those of the most productive mid-sized lake (in the ELA lakes, $1.8 < A_d/A_0 < 15.7$ and $0.044 < A_e/V_e < 0.197$). There is virtually no overlap between Muscow and the ELA lake that best matches the NOLSS design criteria (L.373); and while the complete ELA data set overlaps more with Muscow, the modes are unquestionably separate, implying that intermediate-sized lakes are

consistently more productive than small lakes. But as with the TP data, this evidence is not conclusive, since the NOLSS and ELA study areas differ geologically (Fee and Hecky 1992) and chemically (C. Anema and R. E. Hecky, in prep.); these differences could cause PP to be lower in all ELA lakes than in comparably sized NOLSS lakes.

Conclusion

All measures of phytoplankton productivity (except for the annual rate of PP per square metre of lake surface (Fig. 11D), which is greater in large lakes than in small ones because of the greater number of ice-free days in large lakes) are low and

June-August means

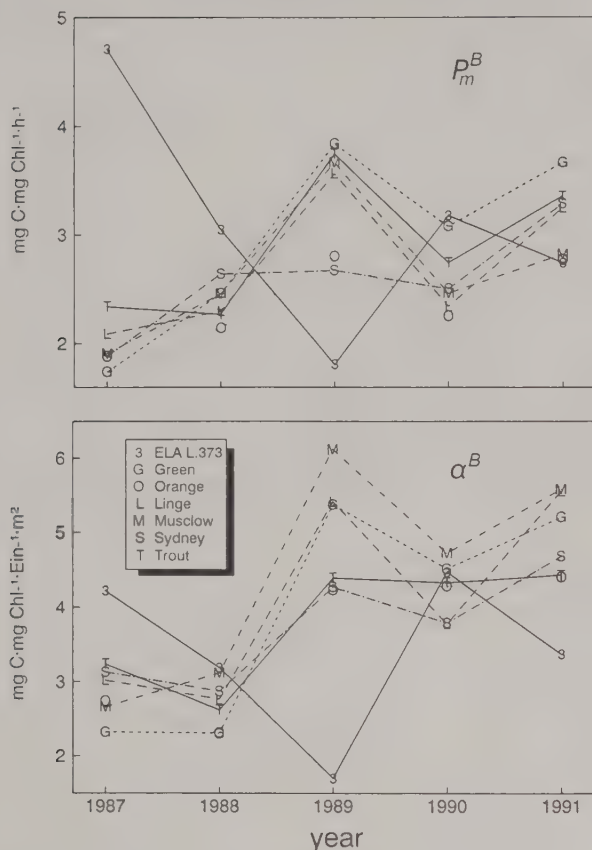


FIG. 7. Means of P_m^B and α^B for the stratified period (June 1 to August 31) plotted as a function of time.

similar in magnitude in very small and very large lakes and increase systematically towards the middle of the size spectrum (Fig. 4, 6, 10). But lake-specific factors (A_d/A_0 , A_e/V_e) along with an index of interannual differences in atmospheric nutrient loading (May–June rainfall) account for most of this variability, suggesting strongly that productivity is independent of lake size. However, there are theoretical reasons (Fig. 13) why productivity should be maximal in intermediate-sized lakes, and other evidence (admittedly less convincing) conflicts with this interpretation (Fig. 16). Thus, we can presently neither accept nor reject the *Size* $\rightarrow$ *Productivity* null hypothesis. This uncertainty could be resolved by measuring PP in a group (six to eight) of intermediate-sized (700–5000 ha), stratified, deep Shield lakes in which A_d/A_0 and A_e/V_e vary widely: if PP were high in all of these lakes, the theoretical model would be validated and the empirical correlation would be proved accidental; on the other hand, if PP varied in accordance with A_d/A_0 and A_e/V_e , the theoretical argument would be disproved. The best place to do this work would be ELA because there would then be no question of geological and methodological commensurability with results from small ELA lakes.

The present results are sufficient to disprove Sterner's (1990) prediction that small lakes are inherently more productive than large ones. It is clear that because of their low levels of turbulence, shallow mixed depths, and short ice-free seasons, small lakes are inherently unproductive, regardless of whether

June-August means

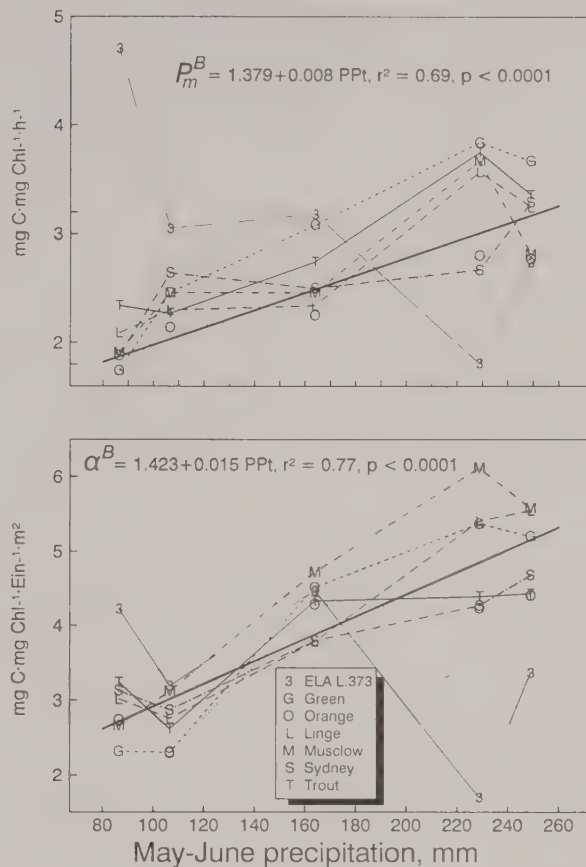


FIG. 8. Means of P_m^B and α^B for the stratified period (June 1 to August 31) plotted as a function of total precipitation during May and June. The heavy straight lines are linear regressions, calculated excluding the ELA L.373 data; the regression equations are given on the figures.

PP is expressed on a daily, ice-free season, areal, or volumetric basis.

Temporal Variability

Another hypothesis that the NOLSS program was designed to test (Fee and Hecky 1992) is: *Natural interannual variability is an inverse function of lake size*. Six years of data is barely enough to address this hypothesis but, with one notable exception, the available data show no decline of variability as lake size increases (Fig. 17). The exception is that chlorophyll was much more variable in the smallest lake (L.373) than elsewhere; this caused P_m^B and α^B in this lake to vary interannually in a very different manner than they did in the other lakes (Fig. 6, 7, 8). The different mixed-layer light climate in small ELA lakes compared with larger lakes (discussed in the next section) may be the cause of this different behaviour. Another factor that must be considered is that ELA chlorophyll samples were analyzed in a different laboratory than the others; thus, we cannot rule out the possibility that this difference is a methodological artifact (currently under investigation).

Interannual differences of chlorophyll and associated photosynthetic rates and parameters in the 2 yr of data available from

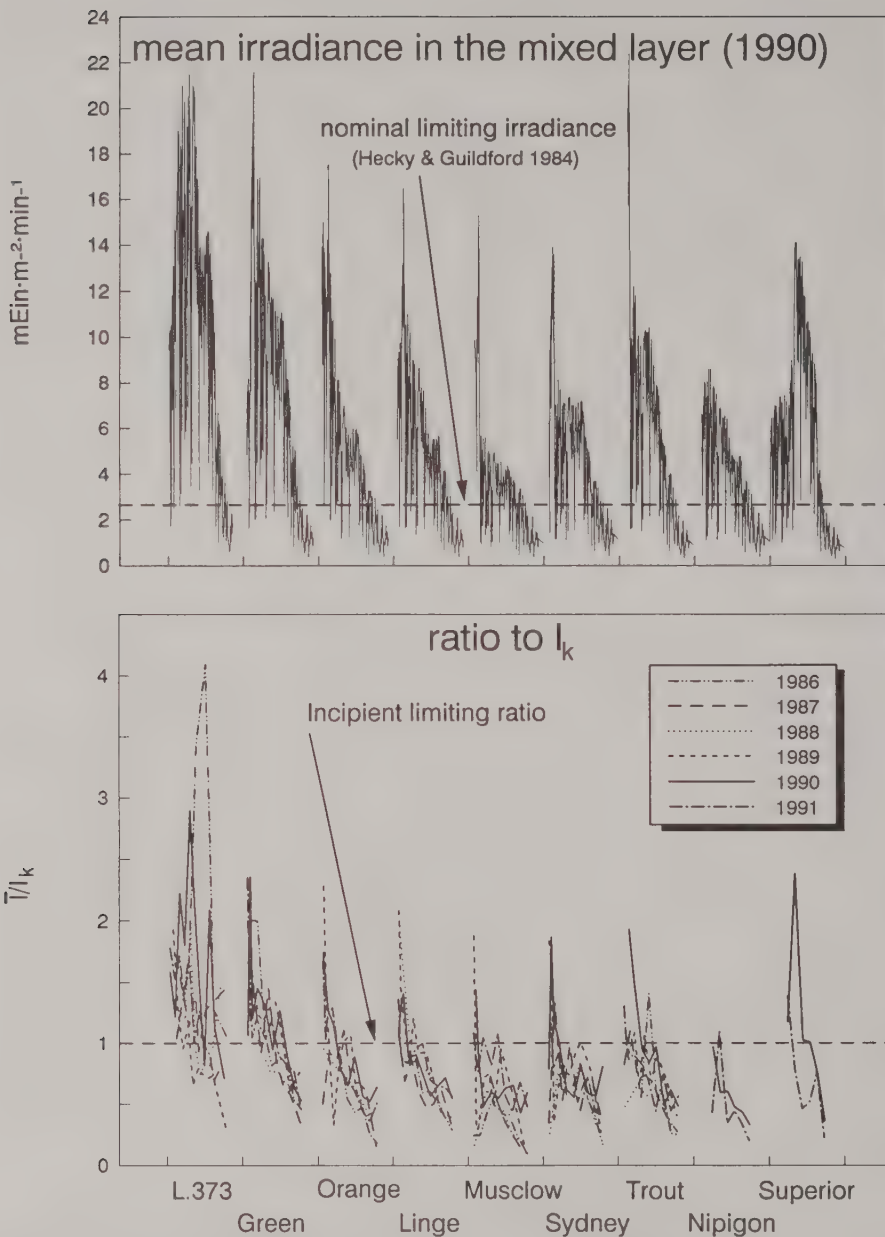


FIG. 9. Top panel: 1990 daily means of PAR in the mixed layer ($\bar{I}$, means for the full 24-h period). The marks on the horizontal axis correspond to May 1 and December 1. The sharp spikes at the start of the year are due to very shallow temporary stratification. Bottom panel: ratio of daily mean PAR in the mixed layer ($\bar{I}$, calculated assuming cloudless surface irradiance) to I_k , the PAR above which photosynthesis is light saturated (see Fig. 1), in lakes varying in size from 29 ha (ELA L.373) to 8 223 600 ha (Superior). Values below the broken line indicate that photosynthesis in the mixed layer is light limited.

Nipigon and Superior (Fig. 4) are less than in the smaller lakes in those years. Although these are scant data, this suggests that interannual variability may decline abruptly with increasing lake size, with the discontinuity occurring at a size larger than the largest lake shown in Fig. 17 (34 700 ha).

Major international efforts are now underway to estimate PP in the world oceans from remotely sensed chlorophyll data. In one approach, numerical models are used to convert chloro-

phyll concentrations into rates of photosynthesis based on specific values of P_m^B and α^B (Platt and Sathyendranath 1988). Regional and seasonal differences in P_m^B and α^B are explicitly included in this model, but interannual variation of these parameters is ignored. The fact that these parameters vary inter-annually by two to three times in freshwaters (Fig. 6; and Fee et al. 1987) indicates that this source of error is potentially important and should be considered by these models. Figure 8

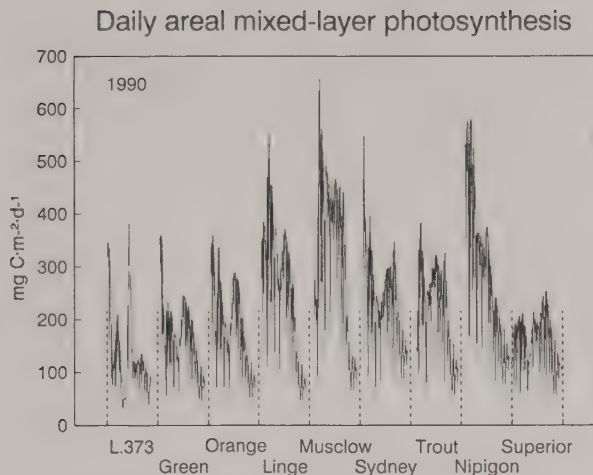


FIG. 10. Daily integral phytoplankton photosynthesis during the ice-free season of 1990 in lakes varying in size from 29 ha (ELA L.373) to 8 223 000 ha (Superior). The marks on the horizontal axis correspond to May 1 and December 1.

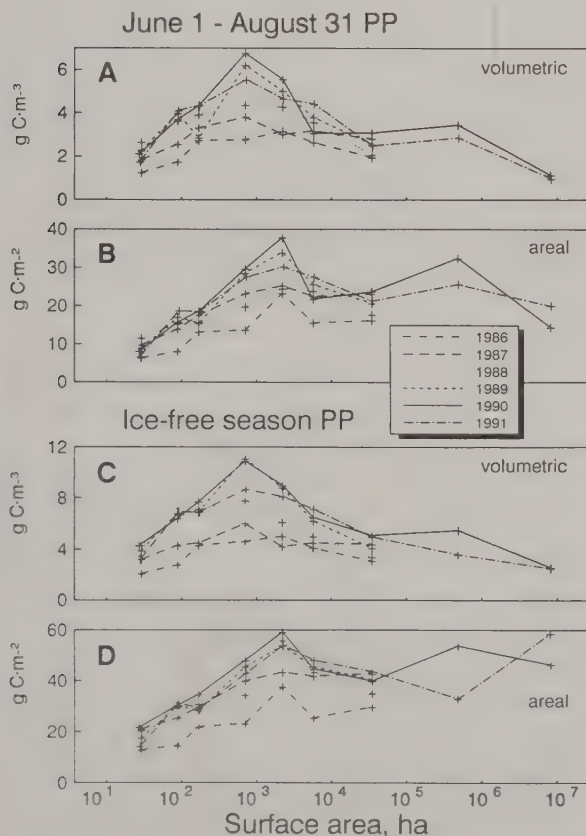


FIG. 11. Mixed-layer phytoplankton photosynthetic rates (A) per cubic metre of the mixed layer and (B) per square metre of lake surface for the stratified period (June 1 to August 31). (C and D) Corresponding totals for the ice-free season. Rates were calculated using actual solar insolation and corrected for the decrease of lake volume with depth (Fee 1980).

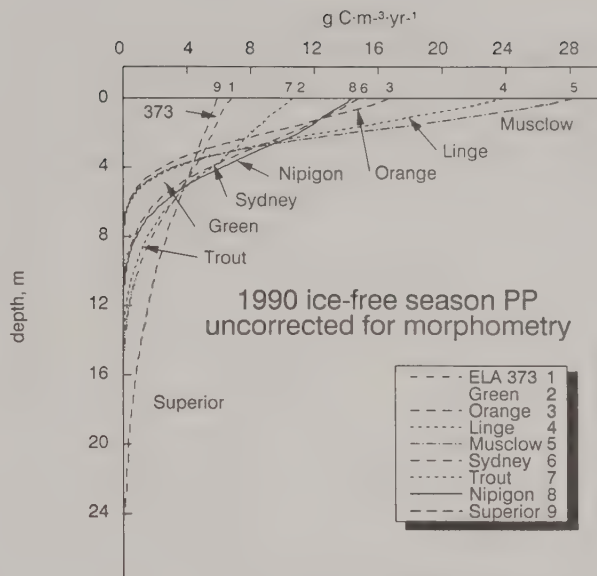


FIG. 12. Ice-free season mixed-layer photosynthesis versus depth profiles in lakes varying in size from 29 ha (ELA L.373) to 8 223 600 ha (Superior) (uncorrected for the decrease of lake area as a function of depth).

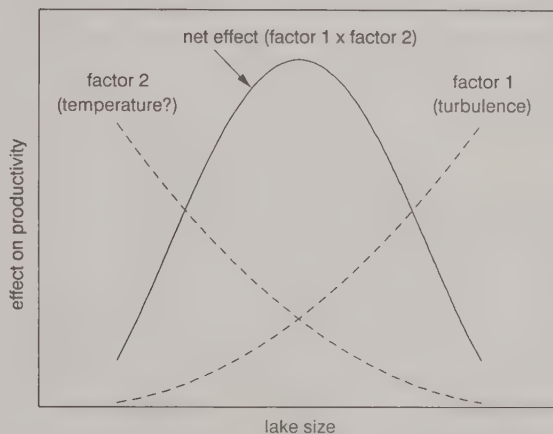


FIG. 13. Simple theoretical model showing why productivity could logically be a function of lake size.

suggests that interannual differences in nutrient loading are responsible for this variability.

Extrapolation of ELA Results to Larger Lakes

ELA lakes have been continuously monitored for more than 20 yr (Schindler et al. 1990). It is important to know how conditions in these small lakes differ from those in larger lakes in order that ELA results can be rigorously extrapolated to larger and more economically important lakes in the region (Fee and Hecky 1992).

One of the most positive findings is that the simple morphometric indices that explain nutrient dynamics in small ELA headwater lakes (A_d/A_0 and A_d/V_d) also apply to larger lakes. This conclusion is based on the fact that together with an index of interannual differences in nutrient loading (May–June pre-

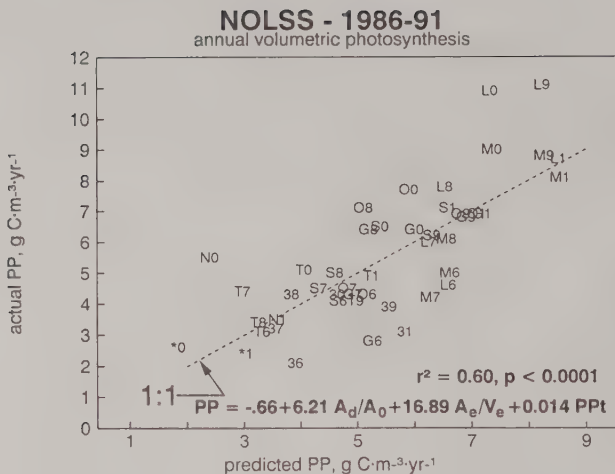


FIG. 14. Statistical relationship between annual PP per cubic metre of the mixed layer and a statistical regression based on A_d/A_0 , A_e/V_e , and total rainfall during May and June. The first character of each symbol is the first letter of the lake name (except for Superior, which is denoted by an asterisk); the second character is the year (6 = 1986, . . . , 1 = 1991).

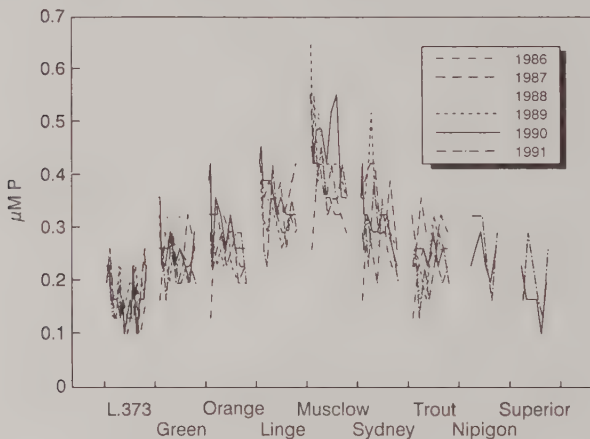


FIG. 15. Variations of total phosphorus during the ice-free season in lakes varying in size from 29 ha (ELA L.373) to 8 223 600 ha (Superior). The marks on the horizontal axis correspond to May 1 and December 1.

cipitation), they explain 60% of the observed variation of PP in the entire lake size series (Fig. 14). This is strong evidence that terrestrial/aquatic interactions are fundamentally similar in all sizes of lakes, and it follows that the nature of these interactions can be elucidated by studies on any size of lake.

Small ELA lakes do have one important functional difference from all larger lakes, viz. $\bar{I}$ in the mixed layers of ELA lakes is much higher than in any lake >100 ha (Fig. 9). The functional importance of this is illustrated in the bottom panel of Fig. 9; $\bar{I}/I_k$ was rarely <1 in lakes <100 ha and rarely >1 in larger lakes, implying that mixed-layer PP is almost always light limited in large lakes but almost never light limited in ELA-sized lakes. Because of the well-known relationship between the depth of the mixed layer and lake size (Patalas 1984; Gorham and Boyce 1989), we expected $\bar{I}$ to decline with increasing lake size, but we were surprised to see it decline so rapidly, and to an essentially constant value, in lakes >100 ha.

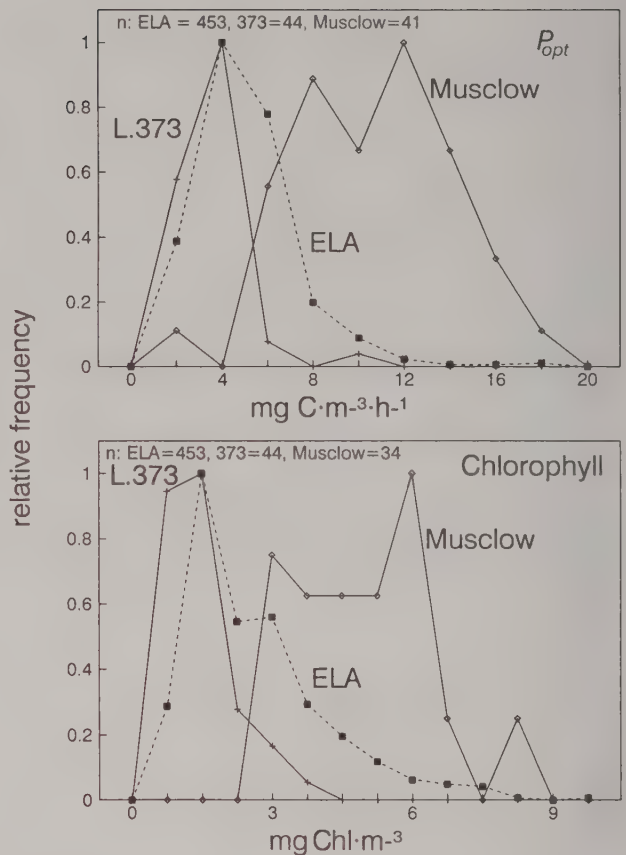


FIG. 16. Frequency distributions of P_{opt} and chlorophyll for the period 1986-91 in the most productive NOLSS lake (Musclove, diamonds), the ELA lake that best matches the NOLSS design (L.373, plus signs), and in all stratified, slow-flushing ELA reference lakes (squares).

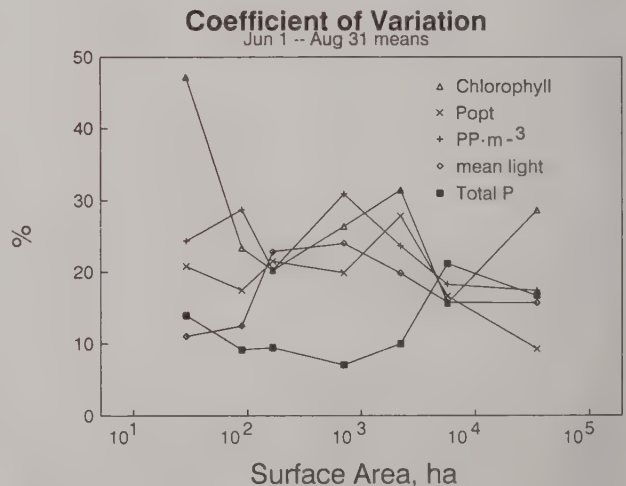


FIG. 17. Coefficients of variation of summer (June 1 to August 31) mean values of chlorophyll, P_{opt} , mixed-layer PP per square metre of lake surface, PP per cubic metre of the mixed-layer, and $\bar{I}$ for the years 1986-91.

This happens because the trend for larger lakes to have deeper mixed-layer depths is opposed by the trend for larger lakes to be more transparent.

Because algae grown in low-light environments are less nutrient deficient than those living in well-lit environments (Healey 1985; Hecky and Guildford 1984), algal populations in small lakes should be more nutrient deficient than those in larger lakes. The extremely low turbulence levels typical of very small lakes (Quay et al. 1980) should exacerbate this by restricting nutrient availability (discussed previously). Actual nutrient deficiency measurements do indeed show that algae in small ELA lakes are extremely nutrient deficient (Healey and Hendzel 1980; L. L. Hendzel, unpubl.), especially in comparison with larger lakes (S. J. Guildford and L. L. Hendzel, in prep.). Consequently, any ELA result that depends on the nutrient deficiency status of the phytoplankton could give inaccurate predictions if extrapolated to larger lakes.

Eutrophication is the best known example of such a phenomenon. ELA was originally established to investigate the causes of eutrophication, especially in the St. Lawrence Great Lakes (Johnson and Vallentyne 1971), and ELA whole-lake nutrient addition experiments showed unequivocally that phytoplankton biomass and productivity in these small lakes are functions of phosphorus loading (Schindler 1977, 1988; Fee 1979). These results were interpreted as strongly supporting the then-controversial policy of reducing phosphorus inputs to the Great Lakes, the expectation being that this would result in substantial declines in phytoplankton biomass in these lakes. But 15 yr of reduced phosphorus loading to Lake Ontario had no significant effect on offshore chlorophyll concentrations (Stevens and Neilson 1987). Similar negative responses of offshore phytoplankton biomass to reduced phosphorus loading have been reported for other large lakes (e.g. Kootenay Lake, Daley and Pick 1990; Lake Constance, Tilzer et al. 1991). We suggest that differences in the mixed-layer light climate between small and large lakes are at least partially responsible for this.

Nevertheless, we believe that reducing phosphorus loadings to these larger lakes was correct policy. Algal biomasses declined in the nearshore zones of Lakes Ontario and Constance (Painter and Kamaitis 1987; H. Müller, cited in Tilzer et al. 1991), where eutrophication problems were most severe, and where most humans interact with these lakes. Further, reduced phosphorus inputs did result in reduced algal biomasses in offshore Lake Erie (El-Shaarawi 1987). These results are in accord with the interpretation presented above because the inshore zones of large lakes are isolated from offshore zones for extended periods in the spring by a "thermal bar" (Boyce et al. 1989) and have light climates more similar to small lakes than to the adjacent offshore zones (Lean et al. 1987), and Lake Erie is so shallow that it is functionally a "small" lake (Mortimer 1987). Thus, even if the effect of lake size on the nutrient status of phytoplankton had been fully understood at the time that the issue of controlling phosphorus inputs to the Great Lakes was being debated, ELA results would have supported the action that was taken because the most heavily impacted parts of the Great Lakes are physically similar to small ELA lakes. There would not have been an expectation, however, that offshore phytoplankton biomasses in the fully stratified Great Lakes would decline. The important point is that limnological processes at the microscale of phytoplankton growth are functions of lake size and that this information should be taken into account when applying experimental results obtained from small lakes to large ones.

Another significant difference between ELA lakes and all larger lakes is that in any given year, the photosynthesis parameters P_m^B and α^B were similar in magnitude in all lakes *except* the ELA lake (Fig. 6, 7, 8). The reason(s) for this disparate behaviour are unclear. As previously mentioned, the ELA samples were processed in a different laboratory than those from the other lakes, suggesting the possibility of systematic errors (now under investigation). It is also possible that the fundamental difference in light climate between small ELA lakes and larger lakes (just discussed) causes ELA lakes to respond differently to the same meteorological forcing. Whatever the cause, it is clear that in any given year, P_m^B and α^B measured in any lake >100 ha can be extrapolated to all lakes in the region *except ELA-sized lakes* in order to estimate phytoplankton photosynthesis from chlorophyll concentration data.

Selection of Lakes for Interregional Comparisons

A goal of NOLSS is to determine what kind of lake would be optimal for interregional comparison studies (Fee and Hecky 1992). The results presented here suggest that a deep (well-stratified), slow-flushing (>5 yr) lake between 100 and 300 ha has the best combination of characteristics for this purpose. Such a lake is still small enough that it is logistically feasible to obtain whole-lake input/output budgets of important chemical substances, yet it is large enough to avoid the high $\bar{I}$ values that occur only in lakes <100 ha (Fig. 6, 9). Finally, lakes of this size on the Shield contain an average of eight fish species (Matuszek and Beggs 1988), which is enough to include at least one representative at all trophic levels; smaller (ELA-size) lakes frequently lack important components in the fish fauna (K. Mills, pers. comm.).

Predicting Effects of Global Warming

A topical issue that NOLSS was designed to address is predicting the effects of climate change on the biological properties of lakes (Fee and Hecky 1992). The results shown in Fig. 3, 4, and 11 show that temperature per se is unrelated to productivity: the two years with the lowest average temperatures were, respectively, the least (1986) and most (1990) productive, while the warmest year (1988) had average PP. Another fact that bolsters this conclusion is that temperature monotonically decreased with increasing lake size (Fig. 3), but productivity was low and similar in magnitude in lakes at the two extremes of the size spectrum (Fig. 4).

On the other hand, there was strong evidence of a link between productivity and rainfall: the three most productive years (1989–91) were the years with highest early summer precipitation. The implication is that if climate change alters the flux of nutrients to lakes, it will affect their productivity. In order to predict the magnitude of these effects, the amount and chemical composition of precipitation under future climates must be known.

One direct effect of climate change on phytoplankton photosynthesis that can be predicted from the presently available data is how the longer ice-free season that would result from climatic warming would change total annual PP. This can be determined by calculating PP with the programs of Fee (1990) after increasing the intervals between sampling dates in order to simulate a longer ice-free season. Because it is presently uncertain how climate warming would affect dates of ice-out and ice-on, we made this calculation twice: first, the ice-free season was made 1 mo longer in the spring and 1 mo longer in the fall; second, the ice-free season was made 6 wk longer in

the spring and was left unchanged in the fall. Both these scenarios resulted in mixed-layer annual PP about 25% higher than current values in all sizes of lakes (actual figures varied between 22 and 28%, with no apparent lake size effect). The only exception was Lake Superior, where PP was unchanged because this lake is already ice-free for 12 mo.

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Cory Anema and Bob Hecky collected most samples on the NOLSS lakes. Paul Cooley assisted them and played a central role in field work on Lakes Nipigon and Superior. Work on Nipigon and Superior was supported by Great Lakes Action Plan (GLAP) funds and was facilitated by Dave Pugh (Ontario Ministry of the Environment) and Rick Salmon (Ontario Ministry of Natural Resources). Bob Hecky, John Rudd, Ray Hesslein, Gordon Koshinsky, Carol Kelly, and Dave Schindler provided useful comments on early drafts of the manuscript.

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Morphometry and Gonad Maturity of Male Snow Crab, *Chionoecetes opilio*

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The relationship between chela height (CH) and carapace width (CW) of male snow crab, *Chionoecetes opilio*, goes through three allometric stages. The "immature stage" (mostly <34 mm CW) evolves into a "juvenile stage" (34–120 mm CW) through a "juvenile molt" defining a change in allometry marked by an angular point around 34 mm CW. Fifty percent of males reach gonad maturity, defined by the presence of spermatophores inside the vasa deferentia, at an estimated size of 34 mm CW. The third allometric stage, "morphometrically mature," is separated from the juvenile stage by a "molt to morphometric maturity" at sizes ranging from 50 to 120 mm CW. Juvenile males have smaller claws than morphometrically mature males of the same size. This secondary sexual character is justified by a specific behavior of the males holding the pereopods of the female in one chela during precopulatory embrace. Male snow crab efficiently mate in nature with intermolt multiparous females only after reaching morphometric maturity. Therefore, the presence of spermatophores is not the sole determinant factor necessary for male copulation. Juvenile males larger than the minimum legal size of 95 mm CW are harvestable before they may efficiently mate.

La relation morphométrique entre la hauteur de la pince (CH) et la largeur de la carapace (CW) des mâles chez le crabe des neiges, *Chionoecetes opilio*, évolue selon trois phases d'allométrie. La phase «immature» (<34 mm CW) évolue en phase «juvénile» (34 à 120 mm CW) après une «mue juvénile» représentée sur la relation d'allométrie par un point anguleux à 34 mm CW. Cinquante pourcent des crabes mâles atteignent la maturité gonadique, établie par la présence de spermatophores dans les vasa deferentia, à une taille estimée à 34 mm CW. La troisième phase, «morphométriquement matures», est séparée de la phase juvénile par la «mue de maturité morphométrique» à des tailles variant entre 50 et 120 mm CW. Les mâles juvéniles ont, à taille égale, une plus petite pince que les mâles morphométriquement matures. Ce caractère sexuel secondaire est justifié par le comportement du mâle qui tient les péréopodes de la femelle dans ses pince durant l'étreinte prénuptiale. Les crabes des neiges mâles observés dans le milieu naturel ne s'accouplent qu'après différenciation de leur pince (maturité morphométrique). Ainsi, la présence de spermatophores n'est pas le seul facteur déterminant permettant aux mâles de s'accoupler. Les mâles juvéniles de taille supérieure à la taille minimale légale de 95 mm CW sont actuellement légalement pêchable avant d'avoir eu l'opportunité de s'accoupler.

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The snow crab, *Chionoecetes opilio* (Brachyura, Majidae), fishery in Atlantic Canada is regulated by a minimum legal size of 95 mm carapace width (CW). The minimum legal size was thought to protect the reproductive potential of the stock (Watson 1970) because female snow crab never grew larger than 95 mm CW and all male snow crab were thought to reach full maturity between 51 and 72 mm CW. Recent works have suggested that the minimum legal size may not protect the reproductive potential of the stock as efficiently as it was once believed (Comeau 1985; Conan and Comeau 1986; Comeau et al. 1991). Conan and Comeau (1986) indicated that in commercial fisheries, as many as 40% of harvested males could be juvenile, i.e. not fully mature individuals.

The terminology dealing with maturity of majid crabs varies between authors (Table 1). For clarity, the terminology proposed by Conan et al. (1986b) will be used in this paper.

The criteria used for identifying maturity of male *C. opilio* differ between authors and imply distinct interpretations of the life cycle. Watson (1970) mentioned that male *C. opilio* simul-

taneously reach morphometric maturity (defined as a differentiation of the shape of chela) and gonad maturity (production of spermatophores) and that all males larger than 60 mm CW were mature. Conan and Comeau (1986) also reported that spermatophores are present in the vasa deferentia of all males larger than 60 mm CW. However, they further showed that differentiation of a secondary sexual character, the shape of the chela, defined as a molt to "morphometric maturity" occurs at CW ranging from 60 to 120 mm for different individuals. They suggested that the differentiation of the shape of the chela is an adaptation to hold the female during precopulatory embrace (Fig. 1). They observed in aquaria that juvenile males, of identical or larger sizes than their mature counterparts, would not develop precopulatory behavior when confronted with multiparous females, i.e. hard-shell females having already mated and spawned at least once (Paul 1984).

The observations made by Conan and Comeau (1986), Conan et al. (1988a), and Comeau et al. (1989, 1991) provide an updated interpretation of the life cycle of *C. opilio* that is consistent with the interpretation most generally provided by other authors on other species of majid crabs. Huxley (1924, 1932), Teissier (1928, 1935, 1948, 1955, 1960), Huxley and Teissier

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TABLE 1. Equivalent terminology used to describe morphometric maturity, critical molts, and gonad maturity for the snow crab (*C. opilio*) and other majids.

	<i>Chionoecetes opilio</i>		Other majids	
Morphometric maturity	Conan and Comeau (1986)	Conan et al. (1988b)	Teissier (1935)	Vernet-Cornubert (1958)
Stage 1	Morphometrically immature	Immature	Immature (1st phase)	Juvenile
2	Morphometrically immature	Juvenile	Immature (2nd phase)	Prepubertal
3	Morphometrically mature	Morphometrically mature	Mature	Pubertal
Critical molts				
Stage 1		Juvenile molt	Prepuberty molt	Prepuberty molt
2	Molt to maturity (terminal molt)	Molt to maturity	Puberty molt	Puberty molt
Gonad maturity	Powles (1968)	Watson (1970)		
Stage 1	Immature	Immature		
2	Mature (undeveloped gonad)	Immature		
3	Mature (developing)	Mature (developing)		
4	Mature (ripe)	Mature (ripe)		



FIG. 1. Snow crab (*C. opilio*) mating couple in precopulatory embrace. Underwater photography taken by Gerard Conan at a depth of 30 m in Bonne Bay, 1984.

(1936), and Hartnoll (1963, 1974, 1978, 1982) have shown that a constant allometric relationship appears as a straight line on a logarithmic plot of chela height (CH) versus CW. Starting from the ordinate, three segments can be identified for majid males. The first segment models the immature stage of small crabs and leads, without discontinuity, to an angular point marking a transition to a second segment which models the juvenile stage. The immature and juvenile stages are separated by a "juvenile" molt. Neither Teissier nor Hartnoll provided a functional interpretation of the juvenile stage. The third line segment, identified as representing a morphometrically mature

stage, is parallel to the second, but shifted in ordinate. Watson (1970) implicitly assumed that morphometric maturity is directly associated with gonad maturity for *C. opilio*, although Hartnoll (1965) indicated that some large juvenile males of related majid crabs (*Microphrys bicornutus* and *Mithrax sculptus*) may carry spermatophores before morphometric differentiation of the claw. The discontinuity between the second and the third segments originates from a "molt to maturity" identified by Teissier (1935) as a terminal molt during which the chela increases considerably in volume by one abrupt step and in certain species changes considerably in shape and ornamentation.

tations (Vernet-Cornubert 1958). The molt to maturity may occur over a two- to threefold range in carapace size.

For male *C. opilio* from the southern Gulf of St. Lawrence, Conan and Comeau (1986) demonstrated the existence of a molt functionally equivalent to the "molt to maturity" and identified it as a terminal molt, but did not at the time identify the "juvenile" molt or infer its biological function.

The objectives of the present work are to demonstrate the existence of a juvenile molt in male *C. opilio* and to interpret its biological function in the reproductive process. Further, we interpret the morphometric, gonadal, and behavioral processes leading to maturity of male *C. opilio* in the perspective of fisheries management.

Material and Methods

Sampling Sites and Gear

Male snow crabs were collected using a Bay of Biscay "Bigouden" *Nephrops* 20-m head rope otter trawl with 2.5-cm-mesh cod end in Bonne Bay, Newfoundland (49°32'N, 57°56'W; Fig. 2), at depths ranging from 120 to 140 m in October 1985 and June–July 1990. Bonne Bay is a deep fjord consisting of two basins. The 140-m outer basin, which extends into South Arm, is connected easterly by a narrow and shallow strait to a second 220-m-deep basin, the East Arm. The Bonne Bay snow crab population remains below the thermocline and is geographically isolated from the Gulf of St. Lawrence fishing grounds by a shallow 50-m glacial sill at the entrance of the bay. Snow crabs are not commercially exploited in Bonne Bay (Taylor et al. 1985) and may show characteristics of a population in its initial state, prior to any commercial fishing. Snow crabs were sampled from the outer basin (Fig. 2). A reference sample was also taken for observation of vasa deferentia on the fishing grounds of baie des Chaleurs (48°03'N, 65°05'W) at a depth of 80 m on November 28, 1984.

Biological Observations

Biometric measurements on CW and CH were made to the nearest 0.1 mm using a modified vernier caliper (Watson and Wells 1970). CW and CH were used respectively as an index for size and for secondary sexual character development, as recommended by Conan and Comeau (1986).

All males measured in 1985 were dissected, and squashes of their right vas deferens were examined under light transmission microscope, at magnifications of 100× and 250×, for presence of spermatophores.

In order to quantitatively study the vasa deferentia condition, a vaso-somatic index (VSI) was defined as the ratio of the wet weight of the vasa deferentia to the wet weight of the whole animal. A total of 23 new-shell (shell not fouled by epizootes) morphometrically mature males (65–144 mm CW), 26 old-shell (shell heavily fouled by numerous epizootes) morphometrically mature males (62–85 mm CW), and 12 juvenile males (76–101 mm CW) in intermolt (molt stage C determined by the observation of the maxilla; Moriyasu and Mallet 1986) with no missing or regenerated limbs were sampled in November 1984. VSI versus CW were plotted on natural logarithmic (log) scales.

Statistical Analysis of Data

To identify morphometric maturity, we used a bivariate discriminant function calculated by Comeau et al. (1991), as described in Conan and Comeau (1986), from a sample of males taken during the 1989 Bonne Bay trawl survey.

The individuals identified as nonmorphometrically mature in the 1985 survey were reexamined to detect possible immature and juvenile stages. All crabs were independently classified into two categories using a criterion of presence or absence of spermatophores in the vasa deferentia. The size frequency histograms specific to each category were examined. In order to

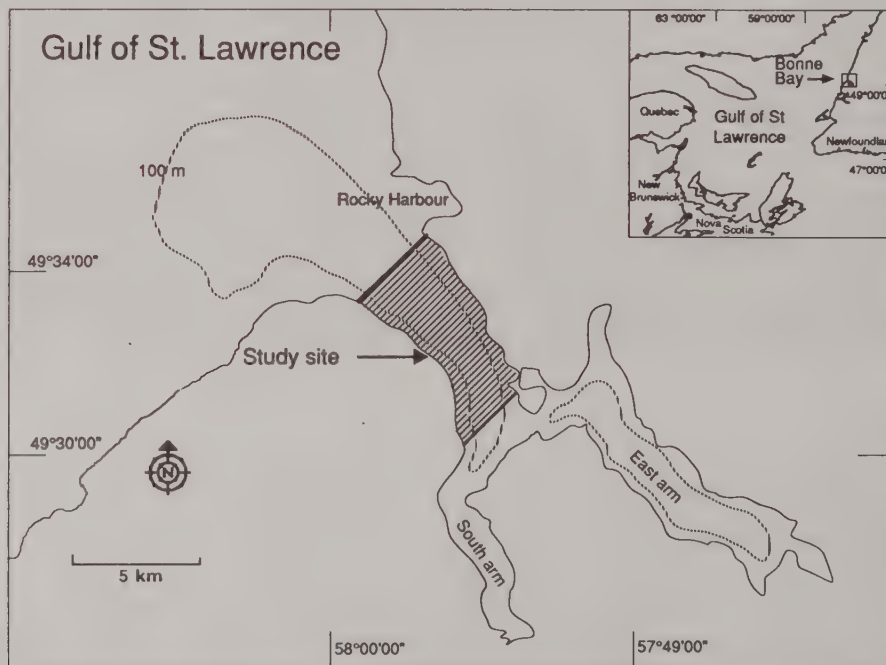


FIG. 2. Map of the fjord of Bonne Bay, Newfoundland, with 100-m depth contour and indicating the site (hatched) where snow crab (*C. opilio*) were collected.

facilitate statistical analysis and conform with standard techniques of comparison of regression lines (Snedecor and Cochran 1980; Gaertner and Laloé 1986), predictive regression lines were fitted by least squares to the log-transformed data separately for each group contrasted, as a substitute for functional regression lines. The estimate of the point of intersection for two regression lines calculated from predictive regression lines would, in this case, differ only slightly from the intersection of equivalent functional regression lines due to high correlations (>0.97) in the allometric relationships of log CH versus log CW. An analysis of variance (ANOVA) was used to test for differences between the regression lines (Snedecor and Cochran 1980), if the residual mean squares were homogeneous (F -test). The point of intersection for the two regression lines was calculated, if the ANOVA showed a significant difference between the slopes, using the equation

$$K_{CW} = \frac{b_2 - b_1}{a_2 - a_1}$$

where K_{CW} is the carapace width at the point of intersection, b_1 and a_1 are, respectively, the y -intercept and the slope of group 1, and b_2 and a_2 are, respectively, the y -intercept and the slope of group 2. The differences between regression lines were tested and the position of the angular point was calculated by the method described by Gaertner and Laloé (1986).

The proportion of individuals bearing mature gonads among the males at a given size was modelled by a logistic curve. Mature gonads are identified by the presence of spermatophores in the vasa deferentia. Data were regrouped into 1-mm CW size-classes. The logistic equation reparameterized as in Conan (1987) is

$$P = \frac{1}{1 + \exp(-4S(CW - CW_{50}))}$$

where P is the proportion of individuals with mature gonads, CW is the carapace width, CW_{50} is the abscissa of the inflection point or size at which 50% of crabs have spermatophores in their vasa deferentia, and S is the slope of the tangent to the maturity curve at the inflection point

$$S = \left(\frac{dP}{dCW} \right)_{CW_{50}}$$

The equations were fitted on untransformed data using Marquadt's iterative nonlinear least square algorithm. The initial estimates were obtained from a regression using the linear transformed equation $y = \log(1/P - 1)$.

Results

Morphometric Maturity

A total of 605 and 1354 male snow crabs were collected by trawl in Bonne Bay in 1985 and 1990, respectively (Fig. 3). Two swarms of points with parallel major axes are identifiable in the graphs of log CH versus log CW (Fig. 4). In 1985, the upper swarm of points, representing the morphometrically mature males (Conan and Comeau 1986), overlaps only slightly (from 57.1 mm CW to 59.5 mm CW) with the lower swarm of points, representing immature and juvenile males (Fig. 4A). In 1990, the two swarms widely overlap (from 51.0 mm CW to 116.6 mm CW; Fig. 4B).

A cutting line based on a bivariate discriminant function is considerably more efficient (99%; Comeau et al. 1991) than a

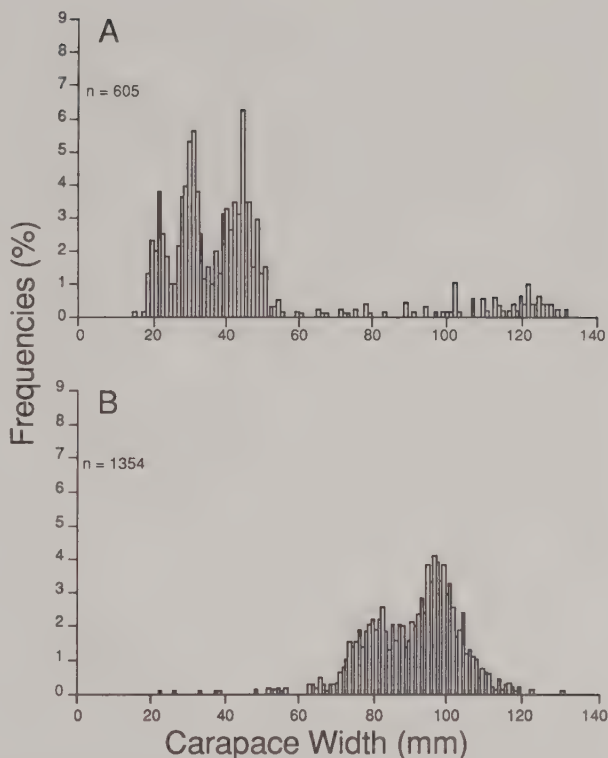


FIG. 3. Size frequency distributions for male snow crab (*C. opilio*) caught by *Nephrops* trawl in Bonne Bay in (A) 1985 and (B) 1990. Total number of individuals (n) collected in both years is indicated.

discriminant technique based on a univariate cutting point criteria. However, CH is more efficient than CW for separating males into juvenile males and morphometrically mature males, as shown in Fig. 4B. By switching from the existing minimum legal size of 95 mm CW to a corresponding 20 mm CH, the proportion of juvenile males retained for sale would have decreased by 26% (from 36 to 10%), while the proportion of morphometrically mature males would have increased by 15% (from 53 to 68%).

Observation of the Vasa Deferentia

Three stages of vasa deferentia development were observed for males: (1) stage I — the vasa deferentia are not morphologically differentiated. (2) stage II — the vasa deferentia are morphologically differentiated, but no spermatophores are observed. The vasa deferentia are translucent and appear as straight tubes. (3) stage III — the vasa deferentia are opaque and their shape varies from a straight tube to a strongly coiled mass. Spermatophores are present in the vasa deferentia.

The size distribution of stage I ranged from 15.1 mm CW to 33.6 mm CW (Fig. 5A) and generated a mode at 22 mm CW in the overall size distribution (Fig. 3A). The size distribution of stage II ranged from 23.4 mm CW to 37.4 mm CW (Fig. 5B) and generated a mode at 31 mm CW in the overall size distribution (Fig. 3A). The size distribution of stage III ranged from 31.3 mm CW to 59.5 mm CW (Fig. 5C) for the nonmorphometrically mature males and generated a mode at 44 mm CW in the overall size distribution (Fig. 3A). All morphometrically mature males determined by the discriminant analysis had stage III vasa deferentia.

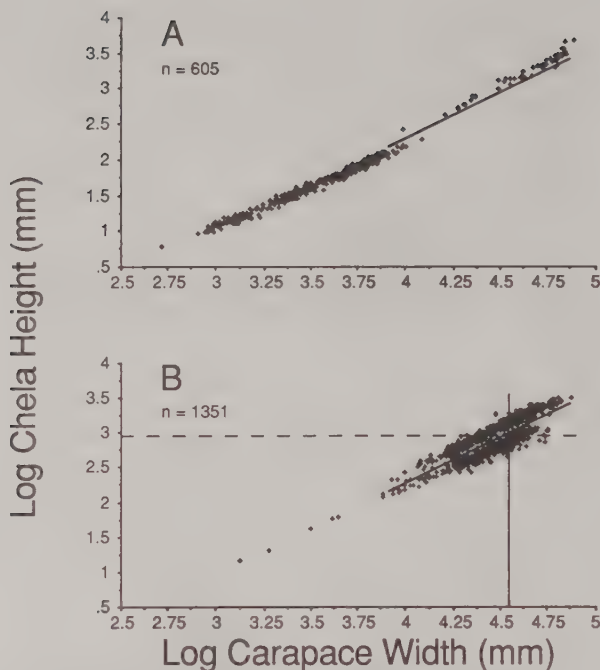


FIG. 4. Relationship between log chela height and log carapace width for male snow crab (*C. opilio*) collected by trawl in Bonne Bay in (A) 1985 and (B) 1990. The cutting line ($\log CH = -2.94 + 1.3 \log CW$) is from a discriminant analysis calculated by Comeau et al. (1991); the upper swarm of points represent the morphometrically mature males and the lower swarm of points represent the immature-juvenile males. In 1990, by switching from the existing minimum legal size of 95 mm CW to an equivalent 20 mm CH, the proportion of juvenile males retained would have decreased by 26% (from 36 to 10%), while the proportion of morphometrically mature males would have increased by 15% (from 53 to 68%).

The morphometrically mature males' VSI was inversely correlated with CW (Fig. 6). Although the size range is small for the old-shell males and the inverse relationship less obvious, the relationships between VSI and CW do not differ significantly ($p = 0.2$) between new-shell and old-shell morphometrically mature individuals. The VSI was significantly ($p < 0.05$) lower for juvenile males compared with morphometrically mature males and was not correlated with CW (Fig. 6).

Gonad Maturity

The logistic maturity curve (Fig. 7) indicates that 50% of male individuals reach gonad maturity as defined by the presence of spermatophores, at 34.2 mm CW.

The plot of log CH versus log CW for immature-juvenile males (Fig. 8) reveals that two distinct intersecting allometric lines may provide a better fit than a single one. A regression line was fitted to each of the sets of points identified by the criterion absence (stages I and II; Fig. 5A and 5B) or presence (stage III; Fig. 5C) of spermatophores in the vasa deferentia. These regression lines differ in slope ($p < 0.05$) when compared by ANOVA. A single regression does not provide as good a fit as $p < 0.05$ when tested by Gaertner and Lalo  s (1986) method. The two regression lines intersect at a CW value $K_{CW} = 34.0$ mm, thus separating an immature from a juvenile stage (Fig. 8).

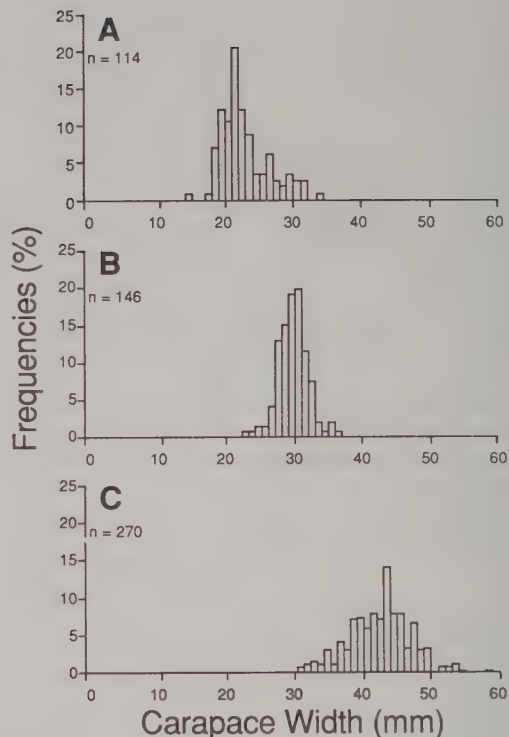


FIG. 5. Size frequency distributions for immature and juvenile male snow crab (*C. opilio*) of the three stages of vasa deferentia development observed in Bonne Bay in 1985: (A) stage I (vasa deferentia not differentiated), (B) stage II (vasa deferentia morphologically differentiated, but no spermatophores are observed), and (C) stage III (spermatophores inside the vasa deferentia).

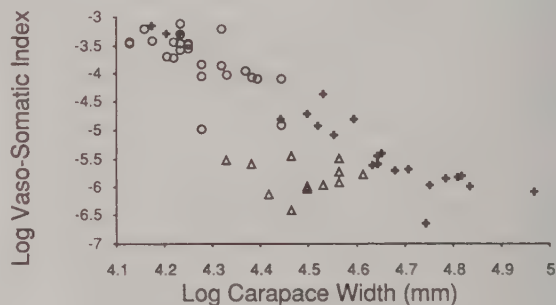


FIG. 6. Relationship between log vaso-somatic index (wet weight of the vasa deferentia/total wet weight of the animal) and log carapace width for juvenile (Δ ; $n = 12$), new-shell ($+$; $n = 23$), and old-shell ($\circ$; $n = 26$) morphometrically mature snow crab (*C. opilio*) males.

Discussion

Gonad Maturity

The three morphological stages of vasa deferentia development we observed in 1985 by macroscopic and microscopic observations appear to be equivalent to the first three of the four stages defined by Powles (1968) and by Watson (1970) (Table 1). The stages I and II define, respectively, males with undifferentiated vasa deferentia and males with differentiated vasa deferentia but no spermatophores. The third

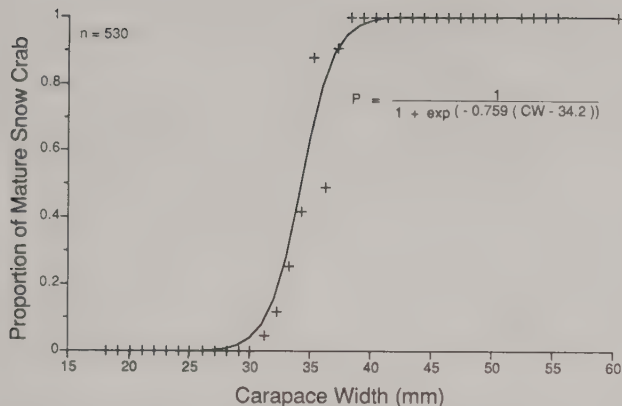


FIG. 7. Relationship between the proportion of males reaching gonad maturity (as defined by the presence of spermatophore in the vasa deferentia) and carapace width of snow crab (*C. opilio*) in Bonne Bay. Fifty percent of male snow crab reached gonad maturity at 34.2 mm CW.

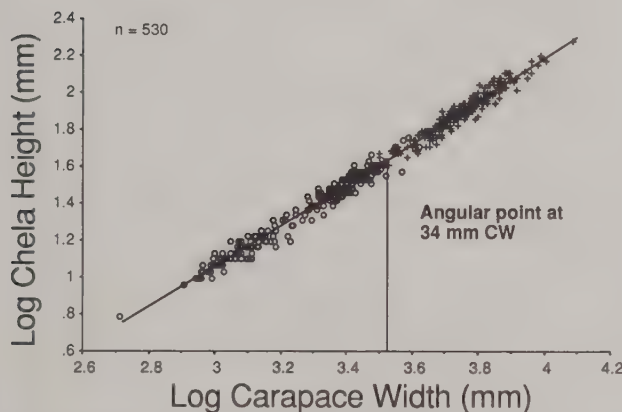


FIG. 8. Relationship between log chela height and log carapace width for male snow crab (*C. opilio*): ○, immature males (stages I and II of the gonad maturity); +, juvenile males (stage III of the gonad maturity). Immature males: $\log CW = -2.23 + 1.09 \log CH$, $n = 260$; juvenile males: $\log CW = -2.52 + 1.18 \log CH$, $n = 270$.

stage regroups males with mature (functional) gonads, and with well-developed, opaque, and convoluted vasa deferentia containing spermatophores.

Powles (1968) and Watson (1970) described the first two stages of vasa deferentia development as "immature," the third as "mature developing," and a fourth stage as "mature ripe" with convoluted, opaque, coiled vasa deferentia extending to fill most of body cavity. Stage 4 "mature ripe" was not observed on morphometrically mature or juvenile males from Bonne Bay in 1985. As shown in Fig. 6, the VSI (wet weight of vasa deferentia/wet total weight) of morphometrically mature males varies inversely in a harvested reference stock with the overall size of the individuals. Powles (1968) and Watson (1970) defined stage 4 solely on the qualitative basis of appearance and volume of the vasa deferentia relative to body cavity. Conan and Elner (R. W. Elner, Environment Canada, P.O. Box 340, Delta, B.C. V4K 3Y3), while diving in Bonne Bay in 1984, observed on the mating grounds a proportion of 1/3 bachelor males to 2/3 males in couples (only

morphometrically mature males were considered) and no solitary females. Stage 4 males were abundant in the Bonne Bay population at that time (pers. obs.). Since 1985, as the population structure evolved, the sex ratio of morphometrically mature males and mature females became unbalanced in favor of females (1:7 in 1985; Comeau et al. 1991) and morphometrically mature male bachelors have been scarce or absent on the Bonne Bay mating grounds (pers. obs.). In a population containing a high proportion of morphometrically mature males, such as in the Gulf of St. Lawrence prior to the present intensive fishing activity (Chiasson et al. 1992), the sex ratio was not unbalanced in favor of females (Brunel 1962, 1963) and stage 4 males were frequently found (Watson 1970). Conversely, in a population intensively harvested, such as presently in the Gulf of St. Lawrence, the number of mature females widely exceeds the number of large morphometrically mature males (Chiasson et al. 1991) and stage 4 males can no longer be found except for very small old-shell males (Y. Chiasson, DFO, P.O. 5030, Moncton, N.B. E1C 9B6). Thus, our interpretation is that Powles' (1968) and Watson's (1970) stage 4 is not a state of maturity of the males, but a state of depletion of the vasa deferentia by spermatophores in small males which are less successful in mating due to competition. Consequently, their vasa deferentia appear more replete than those of larger males.

The three stages of vasa deferentia development are successive and appear to correspond to three successive molt-groups. We suggest that males from stages I and II generate, respectively, modes at 22 mm CW and 31 mm CW in the CW frequency distribution. Males generating a mode at 44 mm CW in the frequency distribution pertain to the first molt-group of stage III. The molt increments presented in this study were approximately 41–42%, which is similar to the percentages of increase between instars found by Robichaud et al. (1989) for small crab caught off Cape Breton in the Gulf of St. Lawrence.

The size at which 50% of males reached their gonad maturity in Bonne Bay (34.24 mm) is much lower than the 57 mm CW observed by Watson (1970), on the basis of the presence of spermatophores in the vasa deferentia in the Gulf of St. Lawrence. A relationship between the development of the vasa deferentia and the presence of molt-groups is given in Kon and Honma (1970) for *C. opilio* in Japan. Male snow crab from Japan reach gonad maturity at instar IX (49.2 mm CW). Spermatophores were not observed by these authors in the vasa deferentia of males during instar VIII (36.8 mm CW). The carapace sizes corresponding to the appearance of spermatophores in the vasa deferentia indicated by Kon and Honma (1970) are somewhat larger than in the Bonne Bay data. Geographical or year-to-year variations may explain the differences in our results.

Relationship between Morphometric Maturity and Gonad Maturity

The allometric relationship between CH and CW for male snow crab is characterized by a shift in ordinate generating a discontinuity between two parallel segments representing immature–juvenile males and morphometrically mature males. These two stages of development are separated by a molt to maturity which is reached over a wide range of sizes for distinct individuals. Watson (1970) observed a small overlap of the two swarms of points from 51 mm CW to 71 mm CW for male snow crab collected from the Gulf of St. Lawrence in 1968 and 1969 prior to the opening of the snow crab fishery. Our data showed that the small overlap of the swarms in the allometric

TABLE 2. Biological characteristics of the terminology used in this paper to describe gonadal, vasa deferentia, and morphometric maturity suggested for snow crab (*C. opilio*) based on Conan et al. (1988b).

Stage of morphometry	Critical molt marking transition to next stage	Size range	Stage of gonad maturity	Vasa deferentia morphology	Presence of spermatophores	Size range
Immature	The "juvenile molt" is reached at a common size for all individuals; it is marked by an angular point separating two distinct allometric relationships	Males <34 mm CW	I	Not morphologically differentiated	No	Males <33 mm CW; major mode at 22 mm CW
			II	Morphologically differentiated as translucent straight tubes	No	22–37 mm CW; major mode at 31 mm CW
Juvenile	The "molt to maturity" is reached at sizes specific to each individual; it is marked by a discontinuity between two distinct allometric relationships	34–120 mm CW (chela nondifferentiated)	III	Opaque with shape varying from straight tube to a strongly coiled mass	Yes	50% maturity at 34 mm CW; all males >37 mm CW
Morphometrically mature	N/A (terminal molt males)	Males >50 mm CW with differentiated chela				

relationship (Fig. 4A) observed in Bonne Bay in 1985 changed into a wide overlap (Fig. 4B) over a period of 5 yr. During that time in Bonne Bay, Comeau et al. (1991) observed a decrease in the number of large morphometrically mature males (due to natural mortality) from 1985 to 1988 followed by the appearance for the first time in 1990 of large juvenile males with carapaces older than 1 yr, i.e. having skipped the spring molt period. The present pattern in Bonne Bay (1990) is quite similar to the pattern of the exploited population in the Gulf of St. Lawrence observed by Conan and Comeau (1986) in 1984. These observations corroborate Waiwood and Elner's (1982) suggestion that the removal of large old crabs would release the snow crab population from a "stagnant" phase into a "dynamic," high-growth phase. Thus, we hypothesize that the removal by fishing, as seen in the Gulf of St. Lawrence, and/or natural mortality, as seen in Bonne Bay, of large morphometrically mature males from the accumulated biomass reduces the mortality of juvenile males, therefore allowing for survival and growth within the lower swarm of points of immature and juvenile morphometric stages. A possible explanation is that at the nonoverlapping swarms stage in the dynamics of a natural population, the structure is maintained in equilibrium by cannibalism of large terminal molt males on juvenile males of similar sizes at the time of their molt.

The shift in ordinate of CH versus CW allometry lines defining two types of male snow crab had been observed but not satisfactorily interpreted by previous authors (Powles 1968; Watson 1970; Coulombe et al. 1985), who associated the "maturity" of gonads with morphometric maturity. Conan and Comeau (1986) provided the functional interpretation of the molt to maturity and its identity with a terminal molt that we presently use. In the present study, we now have shown for *C. opilio* the presence of a third allometric stage at sizes smaller than 34 mm CW prior to gonad development. In other majid

crab species, Teissier (1935) had also detected an "immature" to "juvenile" transition on the basis of morphometry, but had not identified a biological "raison d'être" for this angular point between allometric lines, although he (Teissier 1948, 1960) suspected this transition to correspond to important physiological changes. Hartnoll (1982) mentioned that "the molt to maturity need not coincide with the maturation of the gonads, but it almost invariably indicates entry into the instar in which sexual activity will commence."

We now conclude that maturity of male *C. opilio* is reached through three allometric steps (Table 2) coinciding firstly with differentiation of primary sexual characters (functional gonads) and secondly with differentiation of secondary sexual characters (claw morphometry), related to behavioral (Conan and Comeau 1986) and hormonal changes, as documented by Cormier et al. (1992) on *C. opilio* and indirectly by Homola et al. (1991) on *Libinia emarginata*. These modifications are required by the males to efficiently mate with multiparous females (Conan and Comeau 1986; Ennis et al. 1990; Comeau et al. 1991; Sainte-Marie and Hazel 1992).

Implication for the Fishery

The peculiar majid growth pattern of *C. opilio* has important implications for fishery management. The benefits of setting a minimum legal size should be reconsidered due to the presence of a terminal molt reached by males over a wide range of carapace sizes. The Gulf of St. Lawrence snow crab fishery presently harvests indiscriminately juvenile and morphometrically mature males larger than 95 mm CW (Elner and Robichaud 1986; Comeau and Davidson 1987; Chiasson et al. 1991). In terms of yield-per-recruit, a large proportion of individuals will never grow to the minimum legal size and will never become harvestable. It is advisable for both conservation of stocks (allowing males to reproduce before being caught) and

enhancement of yield (allowing the juvenile males to grow until they reach the terminal molt) to minimize harvesting of juvenile males and maximize harvesting of morphometrically mature males. Due to the particular morphometry of juvenile versus morphometrically mature males, if the minimum harvestable size were defined as a function of chela size rather than the present carapace width, more terminal molt (morphometrically mature) and less preterminal molt (juvenile) crab would be selectively retained as commercial catch (Fig. 4B). An even more efficient, but possibly less practical, alternative would be to use a minimum legal "size" based on two measurements and a bivariate discriminant function (Conan and Comeau 1986). A specially designed electronic gauge allowing direct calculation of a discriminant score CH versus CW would be 99% efficient.

An alternative to a minimum size of either chela or carapace, in order to achieve maximal yield-per-recruit and protect the reproductive potential of the stock, would be to design, as suggested by Conan and Comeau (1986), a type of fishing gear to catch or selectively retain only morphometrically mature animals.

The effects of heavy exploitation of males larger than 95 mm CW on the stock reproductive potential are not yet fully understood. The selective harvesting of large terminal molt males potentially increases efficiency of small terminal molt males to mate by reducing competition pressure from larger dominant terminal molt males. As mentioned by Elner et al. (1986) and by Conan et al. (1988b), this could result in an artificial genetic selection in favor of males reaching terminal molt at small sizes, if an early propensity to molt to morphometric maturity were a genetically inherited character. Fortunately, the storage of spermatophores from large males in the spermatheca of females (Watson 1970) may partially compensate for the depletion of large terminal molt males by fishing.

A detailed knowledge of growth, including the probability for crabs to reach terminal molt as a function of size or age, is required prior to developing a sound yield-per-recruit model. Such a model would allow calculation of parameters such as minimum claw size, effort, catch/biomass ratio, and period for rotation of fishing grounds, optimizing the fishery yield.

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Failure of Dietary Putrescine to Enhance the Growth of Rainbow Trout (*Oncorhynchus mykiss*)

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Reduction of herring meal (to 100 g·kg⁻¹) in rainbow trout (*Oncorhynchus mykiss*) diets and its replacement by soybean and corn gluten meals led to reduced feed intake and growth. The diamine putrescine, when included in calf diets, reduced the effects of antinutritional factors in soybean meal; it also enhanced the growth of chicks. Putrescine (1–4 g·kg⁻¹) added to practical trout diets did not increase feed intake or growth over a 12-wk period. Putrescine concentrations in the trout tissues were not increased by this treatment, nor were the activities of the putrescine and polyamine biosynthetic enzymes ornithine decarboxylase and adenosylmethionine decarboxylase significantly changed. When a higher putrescine concentration (13.3 g·kg⁻¹) was used, in line with the calf diets, it reduced weight gain, feed intake, and gain/feed ratio. Lack of effect of putrescine at the lower concentrations (where it was effective with chicks) was ascribed to the enzyme diamine oxidase, shown to be active in the pyloric ceca and the anterior intestine of the trout. Passage of food through the gastrointestinal tract of trout is about 10-fold longer than in chicks. This may explain the failure of dietary putrescine to elevate tissue putrescine levels in trout.

La réduction de la proportion de farine de hareng (à 100 g·kg⁻¹) dans le régime alimentaire des truites arc-en-ciel (*Oncorhynchus mykiss*) et son remplacement par de la farine de soja et de gluten de maïs a entraîné une réduction de la consommation alimentaire et de la croissance. Lorsqu'elle fait partie du régime alimentaire des veaux, la diamine putrescine réduit les effets des facteurs antinutritionnels de la farine de soja; elle permet également d'améliorer la croissance des poussins. L'ajout de putrescine (1–4 g·kg⁻¹) au régime alimentaire réel des truites n'a pas entraîné d'augmentation de la consommation alimentaire ni de la croissance sur une période de 12 sem. Les concentrations de putrescine dans les tissus des truites n'ont pas été accrues par ce traitement, et les activités des enzymes de biosynthèse de la putrescine et de la polyamine, la décarboxylase ornithine et la décarboxylase adénosylméthionine, n'ont pas changé de façon significative. Lorsqu'on utilisait une concentration de putrescine plus élevée (13,3 g·kg⁻¹), similaire au régime alimentaire des veaux, cela entraînait une réduction du gain de poids, de la consommation alimentaire et du rapport gain de poids/quantité d'aliment. L'absence d'effet de la putrescine à faible concentration (à laquelle elle est efficace chez les poussins) a été imputée à l'enzyme diamine oxydase dont l'activité a été démontrée dans le cecum pylorique et l'intestin antérieur de la truite. Le passage des aliments dans le tractus gastro-intestinal de la truite est environ 10 fois plus long que chez le poussin. Cela peut expliquer l'incapacité de la putrescine diététique à élever les niveaux de putrescine tissulaires chez la truite.

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Commercial fish diets, especially those used in the cultivation of salmonids, contain high concentrations of protein, usually in the range 35–50% of the dry diet. Fishmeal has been a mandatory component of these diets since the inception of large-scale hatchery production with dry diets. Its predominance as an ingredient of fish diets arises from a well-balanced, nearly ideal pattern of indispensable amino acids, a high digestible energy content, and its organoleptic properties with respect to the fish. Most fishmeal is obtained from marine sources and is a limited, if not actually declining, commodity as the oceans continue to be overfished. At the same time, there is an increasing demand for fishmeal for aquaculture and other purposes. Thus, alternatives to fishmeal are seen as desirable, if not actually essential. In addition, the price of fishmeal is high and cost benefits would arise were it replaced by other sources of protein such as oil seed meals or other plant proteins.

Attempts to replace or reduce the fishmeal component of trout diets have, in fact, been made over the past 15 yr or so. They have met with varying degrees of success. Of the plant proteins

tested, most attention has focused on soybean meal. Generally, it has served comparatively well except that high levels of soybean meal and other plant proteins in salmonid diets lead to reduced levels of food intake. Similar observations were made recently on channel catfish (*Ictalurus punctatus*) by Mohsen and Lovell (1990) when diets containing soybean meal (62% in the basal diet) were substituted to a greater or lesser extent with menhaden fishmeal or other animal products.

Recent experiments have shown that feeding putrescine to preruminant calves improved mucosal growth and intestinal nutrient absorption (Grant et al. 1990). When preruminant calves are given milk replacers containing soybean protein, reduced digestion and absorption of nutrients occurs. This is thought to be due to the presence of soybean trypsin inhibitors, allergens, lectins, and other poorly defined factors. Grant et al. (1990) showed that this reduction in assimilation may be partially overcome by the inclusion of putrescine (25 g putrescine dihydrochloride·kg⁻¹) in the diet. In this context, it may be noted that Hendriks et al. (1990) demonstrated binding of soybean agglutinin to brush border membranes in both the proxi-

mal and distal small intestine of Atlantic salmon (*Salmo salar*). This may add to the toxic effect of soybean products in diets for salmonids.

Smith (1990) also observed growth promoting effects from the inclusion of putrescine in chick diets. Using defined diets, in which the protein component was supplied by crystalline amino acids, supplementation with putrescine at concentrations between 1 and 4 g putrescine (free base)·kg⁻¹ led to enhanced weight gain. Higher concentrations of putrescine, up to 10 g·kg⁻¹ in the diet, retarded growth.

The diamine putrescine and the polyamines spermidine and spermine are present in all cells so far examined. They are straight-chain aliphatic amines derived biosynthetically from amino acids. Putrescine is formed by decarboxylation of ornithine; it may be converted to spermidine by the addition of an aminopropyl group provided by the decarboxylation of *S*-adenosylmethionine. Spermidine is converted to spermine in a similar manner.

The polyamines are essential for cell growth, being necessary for the synthesis of proteins and nucleic acids and the formation of hypusine. They affect the activities of certain enzymes and, in addition, putrescine may protect cells from osmotic shock (Pegg 1986).

The object of the present experiment was to examine the effect, on growth and food intake of rainbow trout (*Oncorhynchus mykiss*), of using putrescine to supplement a diet containing substantial amounts of soybean meal and corn gluten meal. In addition, tissue levels of polyamines and activities of putrescine and polyamine biosynthetic enzymes in the trout were measured to help ascertain the fate of the orally administered putrescine.

Materials and Methods

Fish

Two experiments were carried out. In the first, young trout of approximate mean weight 4 g were obtained from Spring Valley Trout Farm, Petersburg, Ont. They were acclimated to laboratory conditions for about 2 wk before being randomly distributed between 18 rectangular fibreglass tanks of 60-L capacity at the rate of 80 fish·tank⁻¹. These tanks comprised six dietary treatments, each replicated three times. Both experiments were conducted according to a randomized complete block design. The tanks were part of a flow-through water system in which water was supplied as a mixture (50:50 blend) of dechlorinated City of Guelph supply and well water. The water temperature was measured daily and thermostatically maintained at 15 ± 1°C; flow rate of water to each tank was approximately 2 L·min⁻¹. Dissolved oxygen content and pH of the water were measured weekly and were in the range 7.5–8.5 mg O₂·L⁻¹ and 7.4–7.7, respectively, throughout the experiment. Photoperiod was a 12-h light, 12-h dark cycle. The fish were fed to satiation three times daily and were weighed collectively every 28 d. At the end of 12 wk the fish were recounted and weighed. There were no mortalities over the course of the growth period.

Samples of liver, kidney, and muscle (from the anteriodorsal region) were taken from fish for measurement of putrescine and polyamine concentrations and activities of ornithine decarboxylase and adenosylmethionine decarboxylase. For each purpose, 12 individuals from each treatment (four from each replicate tank) were anaesthetized in tricaine methanesulfonate (MS 222) and the organs or tissues rapidly dissected, briefly wiped free of any contaminating blood, and

TABLE 1. Composition (g·kg⁻¹) of the experimental diets.

Component	Diets 1–4 and 7–9	Diet 5	Diets 6 and 10
Fishmeal, herring (68% CP) ^a	100	200	300
Corn gluten meal (60% CP)	275	210	150
Soybean meal (48% CP)	360	280	200
Wheat middlings (17% CP)	105	150	195
Vitamin premix ^b	6	6	6
Choline chloride	4	4	4
Ascorbic acid	0.2	0.2	0.2
Mineral premix ^c	2	2	2
Sodium chloride	3	3	3
Fish oil, herring ^d	145	145	140

^aCP, crude protein.

^bSupplied, mg·kg diet⁻¹ except as noted: retinyl acetate, 4000 IU; cholecalciferol, 2500 IU; *dl*- α -tocopheryl acetate, 90 IU; menadione sodium bisulfite, 20; cyanocobalamin, 0.03; biotin, 0.2; folic acid, 10; niacin, 200; calcium D-pantothenate, 70; pyridoxine HCl, 15; riboflavin, 20; thiamin HCl, 20.

^cSupplied, mg·kg diet⁻¹: CuSO₄·5H₂O, 25; FeSO₄·7H₂O, 63; MnSO₄·H₂O, 86; KI, 8; ZnSO₄·H₂O, 144.

^dDeaerated with nitrogen and stabilized with 0.05% ethoxyquin.

immediately frozen in liquid nitrogen. From there they were transferred to a deep freeze at -80°C where they were retained until analyzed (a period of not more than 6 wk).

The second experiment was carried out to ascertain the effects, if any, of higher dietary putrescine concentrations (see below). The fish, of mean initial weight approximately 9 g, were randomly distributed between 12 fibreglass tanks at the rate of 80 fish·tank⁻¹. These tanks comprised four dietary treatments, each replicated three times. Physical conditions in the tank system were as in experiment 1. This experiment lasted 8 wk and diamine oxidase activity was measured in the anterior intestines of the fish in the days immediately following the growth experiment.

No blocking effects were observed in either experiment; thus, no tank effects occurred. This has been the case in many growth experiments carried out in this tank system.

Diets

Three diet formulations were used in experiment 1 (Table 1); they were isonitrogenous (all containing 42% crude protein), all met the indispensable amino acid requirements for salmonids as specified by National Research Council (1981), and were similarly complete in their vitamin and mineral contents. The diets were isoenergetic in terms of digestible energy, all containing 22 g digestible protein·MJ digestible energy⁻¹. They differed in their contents of herring meal, concentrations decreasing from 30% in diet 6 to 10% in diet 1; conversely, diet 1 contained much higher levels of soybean meal and corn gluten meal. Diets 2, 3, and 4 were identical to diet 1 except that they were supplemented with 1, 2, and 4 g putrescine (free base)·kg diet⁻¹, respectively.

The same diet formulations were used in experiment 2 as in experiment 1; diet 7 corresponded exactly with diet 1, diet 8 with diet 3, and diet 10 with diet 6; diet 9 had the same gross composition as diets 1–4, 7, and 8 but was supplemented with 13.3 g putrescine·kg diet⁻¹.

The soybean meal (dehulled, solvent extracted), corn gluten meal, and herring meal were all finely ground (<0.25 mm particle size) prior to use. Putrescine was added to diets 2, 3, 4, 8, and 9 by dissolving the requisite amount of putrescine for a 20-kg batch of diet in 300 mL of deionized water. This putres-

TABLE 2. Concentrations ($\text{mg}\cdot\text{kg}^{-1} \pm \text{SE}$, $n = 6$) of putrescine, spermidine, and spermine in major dietary components and diets.

	Putrescine	Spermidine	Spermine
Herring meal	99.2 $\pm$ 6.8	58.9 $\pm$ 5.2	17.8 $\pm$ 5.6
Soybean meal	148.5 $\pm$ 37.0	306.4 $\pm$ 51.9	36.6 $\pm$ 4.0
Corn gluten meal	53.7 $\pm$ 6.8	50.9 $\pm$ 4.7	47.3 $\pm$ 8.6
Wheat middlings	79.5 $\pm$ 9.3	98.5 $\pm$ 14.2	68.2 $\pm$ 18.7
Diet 1	98.4 $\pm$ 8.8	209.4 $\pm$ 22.7	20.6 $\pm$ 1.3
Diet 2	901.3 $\pm$ 21.2	258.4 $\pm$ 42.1	25.3 $\pm$ 3.0
Diet 3	1717.5 $\pm$ 149.0	225.0 $\pm$ 33.0	25.2 $\pm$ 1.5
Diet 4	3546.1 $\pm$ 205.9	256.8 $\pm$ 38.8	28.9 $\pm$ 2.9
Diet 5	123.3 $\pm$ 13.8	269.1 $\pm$ 30.9	21.8 $\pm$ 2.2
Diet 6	92.2 $\pm$ 8.1	188.6 $\pm$ 14.1	23.8 $\pm$ 2.8
Diet 7	95.4 $\pm$ 7.6	207.8 $\pm$ 18.9	22.4 $\pm$ 1.7
Diet 8	1853.5 $\pm$ 131.3	208.6 $\pm$ 24.6	23.7 $\pm$ 2.5
Diet 9	10933.9 $\pm$ 769.4	219.7 $\pm$ 21.1	20.4 $\pm$ 2.1
Diet 10	96.8 $\pm$ 9.4	184.9 $\pm$ 15.3	22.8 $\pm$ 2.4

TABLE 3. Growth, mean feed intake, and feed conversion efficiency of replicate groups of trout given experimental diets for 12 wk (experiment 1).

	Mean final weight (g) ^a	Mean feed intake (g)	Feed conversion efficiency (gain/feed)
Diet 1	58.65 ^a	53.23 ^{ab}	0.96 ^a
Diet 2	59.22 ^a	52.73 ^a	0.97 ^a
Diet 3	59.71 ^a	53.14 ^{ab}	0.99 ^a
Diet 4	59.54 ^a	53.57 ^b	0.98 ^a
Diet 5	69.78 ^b	56.83 ^c	1.07 ^b
Diet 6	75.42 ^c	57.82 ^d	1.18 ^c
Pooled SEM	0.60	0.10	0.01
Honestly significant difference ^b	2.87	0.481	0.04

^aMean initial weight 6.86 ± 0.01 .

^bValues in the same column with different superscripts are significantly different (honestly significant difference, $P < 0.05$).

cine solution was then added dropwise with constant stirring to a portion of the wheat middlings (1.5 kg) in a large Hobart mixer. The wheat middlings were then mixed with the other components of the diets. The complete mixtures were steam pelleted.

Analysis of Polyamines

Liver, kidney, and muscle samples were homogenized for 30 s in an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH) in the proportion 1 part tissue to 8 parts deionized water and the homogenate centrifuged at 20 000 g for 30 min. The supernatant obtained was deproteinized using the Amicon Micropartition System (YMT membrane, centrifuged at 3 000 g for 60 min). Putrescine, spermidine, and spermine in the protein-free supernatants were then simultaneously analyzed as their *N*-heptafluorobutyrylisobutyl derivatives (Lindquist and Maenpaa 1982) using the capillary chromatographic method of Bedford et al. (1987) with electron capture detection.

The same method was used for the measurement of polyamines in major dietary components and in diets except that 1 g of material was used to ensure that the sample was representative.

Enzyme Assays

Ornithine decarboxylase (ODC) and adenosylmethionine decarboxylase (AdoMetDC) activities were measured in liver and kidney as described by Eloranta et al. (1976) except that incubations were for 60 min and were carried out at 15°C. Output of radioactive CO_2 was linear over this time course and was proportional to the amount of supernatant used in the assay. Protein concentrations in the extracts were measured by the method of Lowry et al. (1951).

Diamine oxidase activity was first measured in tissues from different regions of the gastrointestinal tract of trout from a stock tank. Pyloric ceca and portions of the anterior intestine (i.e. that portion immediately behind the pyloric ceca) and of the posterior intestine were dissected immediately following cervical section. They were washed free of any adhering food material with ice-cold 0.9% NaCl, lightly blotted with absorbent tissue, and homogenized in 0.1 M phosphate buffer, pH 7.4 (1 g of tissue to 4 mL of buffer). After centrifuging (20 000 g for 30 min), diamine oxidase activity was measured in the supernatant as described by Tryding and Willert (1968) except that incubations were for 60 min and the assay was terminated by addition of 0.2 mL of aminoguanidine (2×10^{-5} M). The same procedure was adopted for assays on the anterior intestine of trout from experiment 2.

Statistical Methods

Data were analyzed by analysis of variance and the statistical significance of differences between treatment groups was assessed at the 5% level of probability by calculating the honestly significant difference of Tukey (Steel and Torrie 1960).

Results

Among the major components used in the diets, soybean meal contained higher amounts of putrescine and spermidine than the other materials used (Table 2) but the total amounts of putrescine introduced into the complete diets were at least 10-fold lower than the supplementary putrescine added to diet 2 (1 $\text{g}\cdot\text{kg}^{-1}$). The measured concentrations of putrescine in the unsupplemented diets (1, 5, 6, 7, and 10; Table 2) are in line with the amounts expected from the analyses on the major components (about 80 $\text{mg}\cdot\text{kg}^{-1}$). Some losses of supplementary putrescine appear to have occurred during the steam pelleting but, omitting the small amounts of putrescine contributed by

TABLE 4. Growth, mean feed intake, and feed conversion efficiency of replicate groups of trout given experimental diets for 8 wk (experiment 2).

	Mean final weight (g) ^a	Mean feed intake (g)	Feed conversion efficiency (gain/feed)
Diet 7	48.10 ^b	37.8 ^b	1.10 ^b
Diet 8	49.77 ^b	39.6 ^c	1.10 ^b
Diet 9	28.22 ^a	18.5 ^a	0.89 ^a
Diet 10	59.61 ^c	44.1 ^d	1.25 ^c
Pooled SEM	0.56	0.38	0.03
Honestly significant difference ^b	2.54	1.73	0.14

^aMean initial weight 9.49 ± 0.09.

^bValues in the same column with different superscripts are significantly different (honestly significant difference, $P < 0.05$).

the major components, recoveries of supplementary putrescine in diets 2, 3, 4, 8, and 9 were greater than 80% (Table 2). The values given are for free polyamines; extraction of the materials with 10% sulfosalicylic acid, in an attempt to release any bound polyamines, did not give rise to increased concentrations of polyamines in the extracts.

There were no differences in weight gain between diet treatments 1–4 (experiment 1); supplementation with putrescine was without effect. Best growth occurred in the trout fed diet 6 containing 30% herring meal. The trout given diet 5 (20% herring meal) also grew significantly better than those given diets 1–4. Feed consumption was highest in treatment 6; that of treatment 5 was also significantly greater than that of treatments 1–4 (Table 3). The gain/feed ratio of the fish fed diet 6 was significantly better than that of diet 5, which in turn was significantly better than that of the other treatments (Table 3).

Because the inclusion of putrescine in practical diets up to a level of 4 g·kg⁻¹ had remarkably little effect on growth and food conversion, a second experiment was carried out in which a higher level of putrescine (13.3 g·kg⁻¹) was used. At this putrescine concentration, there were marked reductions in growth rate and feed intake (both of about 50%) together with a significant fall in feed conversion efficiency (Table 4). Growth and feed intake of the trout given the diet with 30% herring meal (diet 10) were again superior to those of trout given diets in which this material was replaced by plant proteins (Table 4).

Apart from small differences in kidney putrescine and spermine which did not relate to dietary putrescine intake, there were no differences in putrescine and polyamine concentrations in liver, kidney, and muscle between treatments (Table 5). Addition of putrescine to diets at a level of up to 4 g·kg⁻¹ did not affect tissue putrescine levels or those of polyamines formed from putrescine. Tissue putrescine and polyamine levels were not measured in experiment 2.

There were no effects of dietary treatments on the activities of ODC and of AdoMetDC in liver and kidney of trout (Table 6). Liver samples intended for ODC assay were inadvertently lost. For both enzymes the activities found by us in trout are somewhat similar to those reported by Corti et al. (1987) for sea bass, precise comparison is difficult because these authors carried out their assays at 37°C. The activities are, however, lower than those reported for homeothermic animals (Bedford et al. 1988a, 1988b), presumably because of the different assay temperatures used (15°C for trout and 37°C for chicks).

TABLE 5. Concentrations of polyamines (nmol·g⁻¹, $n = 6$) in certain tissues of trout given diets containing different amounts of putrescine (experiment 1). Values in the same column with different superscripts are significantly different ($P < 0.05$). honestly significant difference: kidney putrescine, 466.96; kidney spermine, 39.49.

Tissue	Diet	Putrescine	Spermidine	Spermine
Liver	1	510.50	34.87	43.00
	2	503.83	37.17	27.67
	3	523.83	47.00	28.83
	4	489.67	36.17	29.87
	5	457.50	39.67	33.00
	6	545.03	31.67	38.67
Pooled SEM		37.64	5.11	4.47
Kidney	1	1565.67 ^a	36.50	37.17 ^{ab}
	2	1253.33 ^{ab}	36.33	34.17 ^{ab}
	3	1022.33 ^b	30.33	45.00 ^{ab}
	4	924.50 ^b	28.00	22.37 ^a
	5	950.67 ^b	27.50	39.17 ^{ab}
	6	937.67 ^b	55.17	63.83 ^b
Pooled SEM		98.31	6.25	8.31
Muscle	1	377.00	27.67	26.33
	2	386.50	36.50	30.33
	3	375.17	20.83	30.67
	4	388.00	26.83	46.83
	5	350.67	31.00	25.83
	6	338.33	25.33	29.83
Pooled SEM		16.18	7.48	4.50

TABLE 6. Activities (pmol CO₂ released·g protein⁻¹·min⁻¹; mean of six measurements per treatment) of S-adenosylmethionine decarboxylase (AdoMetDC) in liver and kidney and of ornithine decarboxylase (ODC) in kidney of trout given diets containing different amounts of putrescine.

	Liver AdoMetDC	Kidney AdoMetDC	Kidney ODC
Diet 1	89.48	116.89	992.76
Diet 2	92.00	89.53	974.37
Diet 3	109.23	99.83	835.93
Diet 4	94.71	140.27	1019.42
Diet 5	94.79	129.16	1863.20
Diet 6	101.07	113.63	1057.12
Pooled SEM	17.12	26.40	331.22

TABLE 7. Activity (μmol putrescine oxidized·g wet tissue⁻¹·h⁻¹) of diamine oxidase in anterior intestine of trout given diets containing different amounts of putrescine.

	Diamine oxidase activity
Diet 7	0.123
Diet 8	0.103
Diet 9	0.137
Diet 10	0.10
Pooled SEM	0.022

Diamine oxidase activity was measured in the anterior intestine of trout from the second experiment. Preliminary experiments on stock trout showed that activity in the pyloric ceca and in the anterior intestine was of similar magnitude and was higher than in the posterior intestine. There were no differences in diamine oxidase activity between treatments in fish from the second experiment; the high dietary putrescine levels did not enhance activity (Table 7).

Discussion

The advantages of fishmeal over soybean meal, corn gluten meal, and other plant proteins as a source of dietary protein for fish have been variously viewed as due to its nearly ideal pattern of indispensable amino acids, its higher digestible energy content (cf. Mohsen and Lovell 1990), or the antinutritional factors present in plant proteins (Tacon and Jackson 1985). Mohsen and Lovell (1990), in fact, concluded from their experiments on channel catfish that the problem is one of palatability. This conclusion would not rule out an effect of antinutritional factors in soybean meal or the possibility that these factors contribute to its lack of palatability.

The observations of Grant et al. (1990) on preruminant calves appeared to offer a means of overcoming this problem, and consequently we examined the effect of orally administered putrescine. The concentrations of putrescine used by us in the first experiment (up to 0.045 M) were lower than those employed by Grant et al. (1990) (0.16 M) because toxic effects had been observed in chicks when dietary putrescine levels exceeded $6 \text{ g} \cdot \text{kg}^{-1}$ (0.068 M) (Smith 1990).

These putrescine concentrations did not enhance the growth of trout given diets in which the main proteinaceous components were soybean meal and corn gluten meal (Table 3). In this respect the results contrasted markedly with the effects seen in chicks and calves. Our results do emphasize that the main effect of increasing the soybean and corn gluten meal contents of trout diets, at the expense of fishmeal, is to reduce feed intake.

Dietary putrescine concentrations used in the second experiment (0.15 M) were comparable with those used by Grant et al. (1990). At this level the characteristic odour of putrescine was very apparent in the diets; food intake, growth rate, and feed conversion efficiency of the trout were all markedly reduced (Table 4). Tissue levels of polyamines in trout from experiment 1 were generally similar to those found by Smith (1990) in chicks. However, at dietary putrescine concentrations of $4 \text{ g} \cdot \text{kg}^{-1}$ or more, Smith (1990) found significant increases in the concentrations of putrescine and other polyamines in liver, kidney, and muscle of chicks, the increase in muscle putrescine concentration being especially striking. No such increases were evident in trout tissues following oral administration of putrescine. This suggested either that dietary putrescine was not entering the body as such or that, if it was assimilated, it was very rapidly oxidized or excreted.

Measurements of diamine oxidase in trout intestine showed appreciable activity in both the pyloric ceca and in the anterior part of the intestine. Activity in the anterior intestine was not significantly increased by feeding diets containing up to 13.3 g putrescine $\cdot \text{kg}^{-1}$ for 8 wk (Table 7). Nevertheless the likelihood is that orally administered putrescine is oxidized by trout before it can be assimilated.

This raises the question as to why oral administration of putrescine is effective in chicks but not in trout. We have found that diamine oxidase activity in chick intestine, measured at 37°C , is not greatly different from that in trout anterior intestine measured at 15°C (unpublished data). The answer may be that passage of food or digesta through the gastrointestinal tract in chicks is rapid, about 4 h (Hill 1971), while in trout, food remains in the gastrointestinal tract for upwards of 36 h (Fange and Grove 1979).

A study on luminal and basolateral uptake of polyamines by rat small intestine has appeared since these experiments were completed (Bardocz et al. 1990). When putrescine was admin-

istered intragastrically, only 29–39% of the label was found in the body 1 h later, and of that, only 11–15% was present as putrescine. Putrescine catabolism was much more pronounced than that of other polyamines. Bardocz et al. (1990) suggested that catabolic breakdown of putrescine by diamine oxidase reflects a normal protective mechanism against nonphysiological concentrations of putrescine.

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Metabolic Stores of Yellow Perch (*Perca flavescens*): Comparison of Populations from an Acidic, Dystrophic Lake and Circumneutral, Mesotrophic Lakes

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Little is known about the animals that occupy naturally acidic habitats. To better understand the physiological state of animals from temperate, naturally acidic systems, we compared metabolite stores and meristics of two yellow perch (*Perca flavescens*) populations in northern Wisconsin. One population originated from a naturally acidic, dystrophic lake (Acid-Lake-Perch, ALP) and had previously been shown to have enhanced tolerance to low pH. The second population came from two nearby interconnected circumneutral, mesotrophic lakes (Neutral-Lake-Perch, NLP). Perch were collected throughout the year to account for seasonal effects and to discern whether patterns of metabolite utilization differed between populations. ALP had smaller livers containing less glycogen and greater muscle glycogen content than NLP. The ALP also had significantly greater liver and visceral lipid contents, and females from this population committed a greater fraction of their body mass to egg production. We interpret these results as indicative of physiological divergence at the population level in yellow perch. These results are discussed as possible products of H⁺-driven changes in metabolism and as possible products of different life history strategies between populations. Our results also show that perch living in acidic, dystrophic Wharton Lake are not acid stressed.

On connaît peu de choses des animaux qui occupent les habitats naturellement acides. En vue de mieux comprendre l'état physiologique des animaux des systèmes tempérés naturellement acides, nous avons comparé les réserves de métabolites et les variations méristiques de deux populations de perche jaune (*Perca flavescens*) du nord du Wisconsin. La première provenait d'un lac dystrophique naturellement acide (perche de lac acide, PLA), dont la meilleure tolérance à des pH faibles avait été précédemment démontrée. La seconde provenait de deux lacs mésotrophiques, interreliés, à peu près neutres et situés à proximité (perche de lac neutre, PLN). Des perches ont été prélevées tout au long de l'année afin de tenir compte des effets saisonniers et de déterminer si les modes d'utilisation des métabolites différaient entre les populations. Les PLA avaient un foie plus petit contenant moins de glycogène et davantage de glycogène dans les muscles que les PLN. Les PLA avaient également une teneur en lipides du foie et des viscères nettement plus grande, et les femelles de cette population consacraient une plus grande proportion de leur masse corporelle à la production d'oeufs. Nous croyons que ces résultats indiquent une divergence physiologique de la population de perches jaunes. Ces résultats sont traités comme des produits possibles de changements du métabolisme provoqués par des ions H⁺, et comme produits possibles de différentes stratégies de cycle de vie entre les populations. Nos résultats indiquent également que les perches qui vivent dans le Lac Wharton (dystrophique et acide) ne sont pas stressées par l'acidité.

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An estimated 3% of the world's surface is covered by acidic freshwaters (Clymo 1984). Despite this abundance of acidic habitats, we know little about the animals that occupy them. There is even less known about hydrogen ion per se as an evolutionary selection pressure. The cultural acidification of freshwaters has generated an interest in the biology of aquatic organisms at low pH, with the resulting work usually focusing on short-term effects. With fish, most of these studies have involved exposing hatchery or aquarium stocks to low-pH conditions for relatively short periods. When feral stocks have been used, they have infrequently originated from naturally acidic waters (see Vinogradov and Komov 1985; Rask and Virtanen 1986; Gonzales and Dunson 1987, 1989; Nelson 1989, 1990 for exceptions). Even rarer are attempts to examine

the biology of animals from naturally acidic habitats in situ (Brown and Jewell 1926; Rahel 1983; Nelson et al. 1988).

Fishes endemic to acidic habitats can be extremely tolerant of pH depressions (Dunson et al. 1977; Dederen et al. 1986; Gonzales and Dunson 1987), and populations of individual species can show increased tolerance if they come from an acidic habitat (Rahel 1983; Valtonen and Laitinen 1988). Preservation of ionic balance (McWilliams 1982; Gonzales and Dunson 1987, 1989), O₂ delivery (Dederen et al. 1986; Nelson et al. 1988), and acid-base balance (Nelson and Mitchell 1992) appears to be important in this increased tolerance, but whether metabolic adjustments are part of this response is unknown.

A major objective of this study was to use storage metabolite levels and meristic measurements to assess the physiological status of one acid-resistant yellow perch (*Perca flavescens*) population (Acid-Lake-Perch, ALP) (Rahel 1983). Values were compared with those from a second population occupying two

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interconnected circumneutral lakes (Neutral-Lake-Perch, NLP) and differences between the two were compared with known perturbations in similar factors from the 'acid-stress' literature.

A second objective was to determine whether metabolic adjustments were found in animals classified as acid tolerant. We compared metabolite stores of ALP and NLP throughout one annual cycle. Temporal variation was included to compare patterns of metabolite storage and utilization between perch populations and to remove, statistically, seasonal influences on the measured components. We also accounted for the potentially important covariates of sex, reproductive state, condition factor, and size in our analysis. Populations of the same species were compared so that observed differences were likely to have arisen from environmental factors, and not from species differences (Huey and Bennett 1986). Although acidity is only one factor that differs between these lake types, it appears to be one of the most critical in structuring fish communities (Magnuson et al. 1989; Tonn et al. 1990).

A final objective was to determine whether differences in metabolite stores between populations of perch were consistent with hypotheses concerning selection for strategies that limit endogenous H^+ production.

Materials and Methods

Yellow perch were samples from one acidic and two circumneutral lakes in Vilas Cty, Wisconsin, approximately bimonthly from October 1985 through August 1986 (Table 1). Physical characteristics of the lakes can be found in Nelson et al. (1988) and chemical composition in Nelson (1989). The acidic lake (Wharton, source for ALP) is geographically isolated from other lakes and fish migration to or from the lake is not currently possible. The two reference lakes (Allequash and Trout, sources for NLP) are connected by a short (1 km), navigable stream. The lakes were chosen on the basis of their geographic proximity (to reduce the possibility of founder effects) and the variety of habitats available to NLP within their system including lotic, deep water oxygenated, summer anoxic, and winter anoxic waters. Capture of perch in Trout Lake was restricted to the south basin where genetic exchange with Allequash Lake is most likely to occur. All fish were captured by angling during daylight hours. Fish were secured as rapidly as possible and

immersed in liquid nitrogen within 7–20 s of being hooked. Fish were sealed in an airtight bag, placed on dry ice, and stored frozen until processed.

Samples were prepared from white muscle, liver, gonad, and viscera. Frozen white muscle was removed by scraping off overlying skin and red muscle and chiseling out a piece of epaxial musculature (~0.5 g). Muscle samples were weighed quickly, placed in individual containers, and kept frozen on dry ice. A single liver sample was chiseled out of the frozen fish and immediately returned to dry ice for later glycogen analysis. Fish were then allowed to thaw sufficiently to remove liver, gonads, and viscera. These were kept on aluminum blocks in contact with dry ice during processing. Liver and gonad tissues were separated into aliquots, weighed, and refrozen on dry ice. Gut contents were removed and the remaining viscera weighed and refrozen. All tissues remained frozen in sealed containers until assayed.

Total protein content was analyzed from epaxial white muscle and gonads. Frozen aliquots were homogenized on ice in a 0.05 M Tris, 0.15 N KCl buffer (pH 7.2). Appropriate dilutions were analyzed for protein according to Bradford (1976) at 595 nm using a Beckman DU-6 spectrophotometer. Standards were prepared from bovine gammaglobulins (Sigma Chem. Co. No. G-7516).

Lipids from epaxial white muscle, gonads, liver, and viscera were extracted using a variation of Radin's (1981) method. Samples were homogenized in hexane-isopropanol (3:2), centrifuged at $5000 \times g$, and the supernatants placed in pretared tubes. Pellets were washed twice, recentrifuged, and all supernatants pooled. Solvent was volatilized by heating to $50^\circ C$ under a nitrogen stream; the tubes were then weighed to 0.01 mg on a Sartorius analytical balance.

Glycogen content was measured in liver and epaxial white muscle. Frozen samples were digested in boiling 30% KOH; glycogen was precipitated with 95% ethanol and centrifuged at $4000 \times g$ and $4^\circ C$. The pellet was then washed with ice-cold distilled water and glycogen reprecipitated with ethanol. Glycogen was acid hydrolyzed and glucose determined with anthrone reagent (Carroll et al. 1956). Optical density at 625 nm was measured in a Beckman DU-6 spectrophotometer using dried glucose as a standard. Glycogen was not measured in muscle collected in October, January, or March.

TABLE 1. Data of capture, size, age, condition factor, and gender of the study fish. Standard deviations are given in parentheses, when appropriate. Condition factor = $\text{mass (g)}/\text{length (cm)}^3 \times 100$.

	Capture data					
	Jan. 2–4, 1986	Mar. 1–2, 1986	May 25–28, 1986	July 5–6, 1986	Aug. 8–10, 1986	Oct. 4–6, 1985
Wharton Lake						
No. of fish	10	8	10	9	8	10
1-m water temp. ($^\circ C$)	1.6	2.6	19	24.3	23.3	9.9
Mean size (g)	31.4 (9.8)	27.2 (9.7)	46.2 (26.3)	35.0 (20.4)	31.1 (12.2)	32.9 (5.8)
Age (yr)	3.6 (0.52)	4.0 (0.19)	4.5 (1.3)	3.9 (1.0)	3.2 (0.89)	3.3 (0.48)
Condition factor	0.94 (0.14)	0.89 (0.08)	0.88 (0.13)	0.87 (0.14)	0.85 (0.09)	1.0 (0.20)
Sex (M, F)	4, 6	3, 5	2, 8	2, 6	1, 6	0, 10
Reference lake						
Lake	Both	Allequash	Both	Trout	Trout	Trout
No. of fish	11	8	10	9	9	8
1-m water temp. ($^\circ C$)	0.9	0.8	21.3, 20.0	21.5	24	11.5
Mean size (g)	41.9 (15.2)	57.0 (26.7)	52.2 (28.3)	45.5 (28.1)	38.8 (28.0)	50.5 (38.7)
Age (yr)	3.1 (0.54)	3.5 (0.76)	3.6 (0.97)	3.6 (1.3)	3.0 (1.4)	3.3 (1.7)
Condition factor	0.91 (0.2)	0.98 (0.11)	0.97 (0.37)	0.86 (0.08)	0.88 (0.11)	0.99 (0.13)
Sex (M, F)	9, 2	3, 5	6, 2	2, 5	4, 4	3, 5

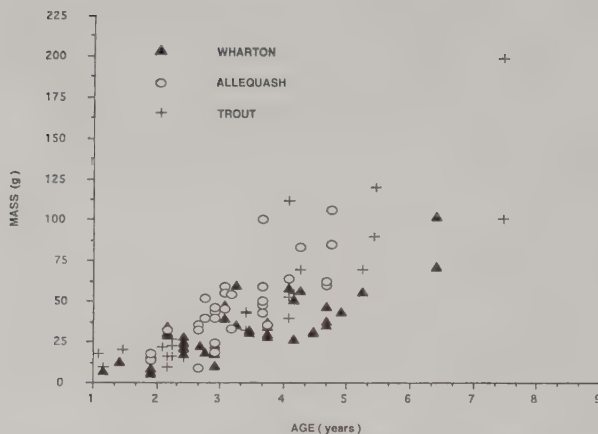


FIG. 1. Fish size at age for 96 yellow perch from three different northwestern Wisconsin lakes. Allequash and Trout lakes are connected by a short, navigable stream; perch from these two lakes actually represent a single population (NLP). Perch from Wharton Lake are ALP.

Relative proportions of red and white muscle in a transverse section were measured optically. A 5-mm cross section was cut from a frozen fish just behind the vent, thawed, immersed in water, and photographed. Photographic slides (35 mm) were projected onto an Apple graphics tablet; total cross-sectional area and red muscle area were measured by digitization of two photographs from each fish.

Maximal enzyme activities (V_{max}) were determined in crude homogenates prepared from epaxial white muscle of fish collected in May 1986. Prewashed frozen muscle was homogenized 1:10 in 0.05 M Tris, 0.15 M KCl (pH 7.2), split into aliquots, and immediately refrozen on dry ice. All enzyme assays were performed within 1 wk of homogenization. Substrate and cofactor concentrations were optimized; therefore, reported activities are indicative of potential flux through a reaction and not actual *in vivo* reaction velocities. Activities were determined from changes in the absorbance of nicotinamide adenine coenzymes at 339 nm and 30°C in a Beckman DU-6 spectrophotometer.

L-Lactic dehydrogenase (EC 1.1.1.27) (LDH) was assayed as a pyruvate reductase after Bergmeyer (1984). Muscle tissue was diluted 1:200 in Tris/KCl (pH 7.2) and a volume fraction (ψ) of 0.016 was used. The assay medium 0.08 M Tris, 0.20 M KCl, 0.2 mM NADH (Sigma No. N 8129), 5 mM pyruvate (Sigma No. P 2256) (pH 7.2) at 30°C. Accuracy was checked with rabbit muscle LDH (Sigma No. L 2500).

Hexokinase (EC 2.7.1.1) (HK) was assayed linked to the glucose 6-phosphate dehydrogenase reaction after Easterby and Qadri (1982). The assay medium contained 32.5 mM glucose, 9.8 mM $MgCl_2$, 19.5 mM Tris (pH 7.5 at 30°C), 0.5 mM NADP (Sigma No. N 3886), 0.25 unit of glucose 6-phosphate dehydrogenase (Sigma No. G 7877), and 5 mM ATP (Sigma No. A 6144) in a total volume of 1 mL. The volume fraction of diluted sample (1:10) was 0.1, and the reaction was initiated with ATP. The appearance of NADPH showed an exponential decay in tissue samples, so the maximal velocity was determined by extrapolating the approximately linear rate over the first 2 min (measured at 10-s intervals) to zero time. Accuracy was checked with Baker's yeast HK (Sigma No. H 5625).

Total phosphorylase (GP) activity (in the presence of AMP) was measured linked to the glucose 6-phosphate dehydrogenase reaction by converting the glucose 1-phosphate product to glu-

cose 6-phosphate (Helmreich and Cori 1964). The assay medium contained 40 mM imidazole, 40 mM KH_2PO_4 (pH 7.5 at 30°C), 1.5 mM Na_2EDTA , 10 mM $MgCl_2$, 1 mM dithiothreitol, 1% glycogen (Sigma No. G 0885), 0.01 mM glucose 1,6-diphosphate, 0.5 mM NADP, 1 mM AMP (Sigma No. A 1752), 0.05 unit of phosphoglucomutase (Sigma No. P 6156), and 0.05 unit of glucose 6-phosphate dehydrogenase. The reaction was initiated with sample supernatant (1:35, volume fraction 0.05) and was linear throughout.

Water content was determined in white muscle, liver when enough was available, and gonad during the reproductive season. Tissues were dried to constant weight for 2 d at 55°C and cooled in a desiccator prior to reweighing.

Ages were estimated by reading scales from 15 fish from each of the two reference lakes (NLP) and 20 fish from Wharton Lake (ALP). Scales were removed from the midline, just posterior to the pectoral (P_1) fin, and read on a microfiche viewer. These data were pooled with age data from an earlier study (Nelson and Magnuson 1987), used to generate a size at age relationship for each population, and from that assign an age to each fish in our data set based on size and a presumed May 1 hatching date (Fig. 1).

Data were analyzed using the general linear models (GLM) procedure of the Statistical Analysis System (SAS Institute 1982). The general model was a two-way unbalanced analysis of variance (ANOVA) with lake and collection date as class variables. Treatment variables also were analyzed separately by sex and reproductive condition. Certain variables were size or condition factor dependent, in which case they were included as covariates and an analysis of covariance (ANCOVA) used as the model. Because freezer storage time significantly affected muscle water content, these data were first regressed on storage time and subsequent analyses performed on the residuals. Analyses were performed on logarithmic transformations of parameters not normally distributed (i.e. ratios). All data and error bars presented graphically and not identified as least squared means are in an untransformed state to facilitate comparison with extant literature. Thus, care should be exercised in drawing statistical conclusions from the figures. Tukey's multiple comparison test was used to compare means, the recommended test for unbalanced designs (SAS Institute 1982). When ANCOVA was used, the standard errors of the least square means were used to assess significant differences between means (Freund and Littell 1981).

Results

Results are summarized in Table 2. Because we are interested primarily in differences between the populations, these are discussed first under any given variable heading, followed by the often more influential collection date and any other significant covariate(s). We have used a decision level probability of 0.05 throughout; the order of progression through the results follow Table 2.

Metabolite Stores

Liver glycogen

Liver glycogen concentration was extremely variable, ranging from 1 to 400 (μ mol glucosyl units/g wet weight). When we considered total liver glycogen, standardized for body mass, NLP had significantly greater total liver glycogen than ALP (Fig. 2a). This interpopulation difference in hepatic glycogen content was observed primarily in females; males from the two

TABLE 2. Summary of experimental results considering population and capture date as the treatment variables with size, condition factor (CF), sex, and the lake from which the reference fish originated as covariates. Decision level = 0.05 throughout. Inter. = interaction term, + = presence of a significant effect, - = insignificant relationship, NA = inappropriate to consider treatment variables individually because of the presence of a significant interaction term. ND = not determined. Wh = fish from naturally acidic Wharton Lake, and Ref = fish from the interconnected reference lakes Trout (Tr) and Allequash (Aq).

Response variable	Main effects				Confounding factors			
	<i>n</i>	Lake	Date	Inter.	Tr ≠ Aq	Size	CF	Sex
Glycogen								
Liver	104	Wh < Ref	+	-	+	-	-	+
Muscle	55	NA	NA	+	+	-	-	-
Lipid								
Visceral	86	Wh > Ref	+	-	-	-	+	+
Liver	102	Wh > Ref	-	-	-	+	-	+
Muscle	107	-	+	-	-	-	-	-
Gonad	53	-	-	-	-	-	-	+
Protein								
Muscle	89	-	+	-	-	-	+	-
Gonad	53	-	+	-	-	-	-	+
Water content								
Liver	85	-	+	-	-	-	-	-
Muscle	108	-	+	-	-	-	-	-
Gonad	53	NA	NA	+	-	-	-	+
Various								
Hepatosomatic index	105	NA	NA	+	-	-	-	+
Gonadosomatic index (♀)	32	Wh > Ref	+	-	-	+	-	NA
Gonadosomatic index (♂)	21	-	+	-	-	-	-	NA
% red muscle	61	-	-	-	-	-	-	ND

populations did not differ significantly in hepatic glycogen content.

Muscle glycogen

Glycogen concentration in muscle was determined by an interaction between collection date and lake origin of the fish. Muscle glycogen was significantly greater in ALP than in NLP, despite being roughly the same in July 1986 (Fig. 2b).

Visceral lipid

Fish from dystrophic Wharton Lake (ALP) generally had more visceral lipid than NLP (Fig. 3a). Lipid amounts never differed significantly within a collection, but ALP always had more visceral lipid than NLP. Visceral lipid levels varied strongly with season; visceral lipid peaked in late May, gradually declined throughout the summer months, and stayed steady and low during winter. Visceral lipid content correlated strongly with condition factor ($P < 0.0001$), confirming the exchangeability of these two parameters in assessing condition of perch. When this strong relationship with condition factor was included as a covariate in an ANCOVA, male ALP had significantly more visceral lipid than male NLP. Female ALP and NLP had similar visceral lipid contents, 13.2 versus 12.7%, respectively, whereas the mean visceral lipid content of male ALP was 11%, compared with 7.3% for NLP males.

Liver lipid

Perch from Wharton Lake (ALP) had greater liver lipid content than NLP ($P < 0.0001$; Fig. 3b). The difference was apparent on all collection dates. Size and sex both influenced liver lipid content. Larger fish tended to have proportionately less liver lipid. Males and immature female ALP always had more liver lipid than NLP, but sexually mature females had similar liver lipid levels regardless of the population from which they originated. Liver lipid content was one of the few variables that did not differ significantly among seasons (Fig. 3b; Table 2).

Muscle lipid

Muscle lipid contents did not differ between ALP and NLP, although mean muscle lipid levels were usually higher in ALP (Table 3). Season influenced muscle lipid levels. Changes in muscle lipid paralleled those of visceral lipid, with a maximum in late May followed by a gradual decline until winter.

Gonad lipid

Gonad lipid was analyzed only for fish collected when they were reproductively active. Lipid contents in developing ovaries and testes were not different between populations.

Muscle protein

Muscle protein content did not differ between perch populations (Table 3). Both season and condition factor influenced muscle protein content. Fish in better condition had more protein in their muscle. Bradford-active protein content increased throughout the summer, declined in October, and stayed low throughout the winter (Table 3).

Gonad protein

Neither ovarian or testicular protein content differed between populations.

Other Measurements

Glycolytic enzymes

White muscle from Allequash Lake perch had greater LDH and HK activities than white muscle from Trout and Wharton Lake perch, as well as greater GP activity than Trout Lake perch muscle (Fig. 4).

Water content

Population origin and season interacted to influence ovarian water content (Table 3). Ovarian water content was steady in NLP, but increased throughout the winter in ALP. Liver and muscle water contents did not differ between populations (Table 3). Water content of muscle was analyzed after statis-

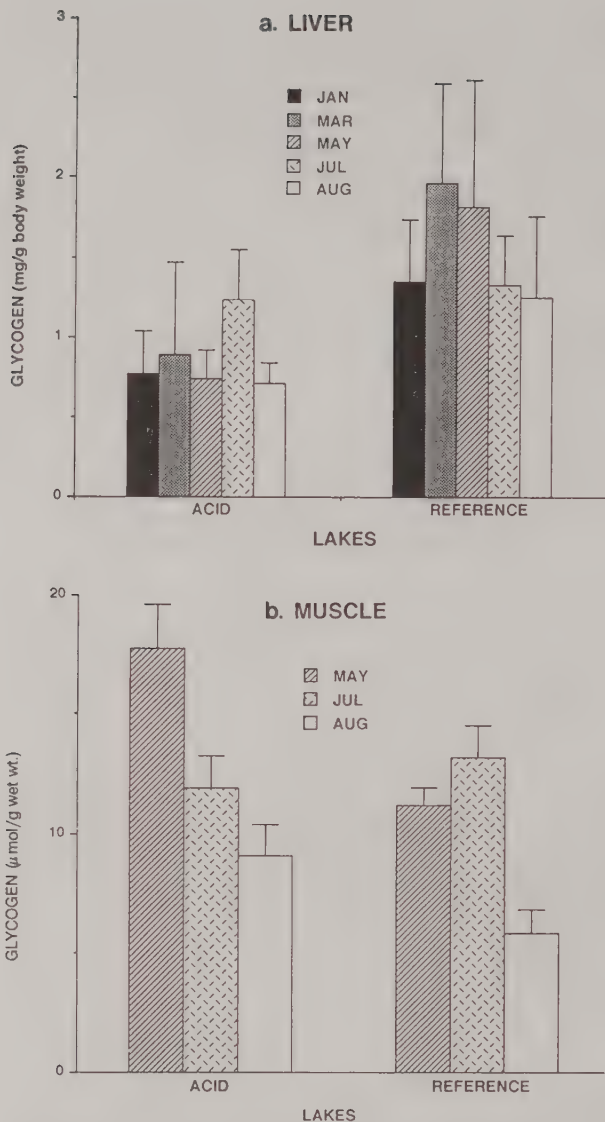


FIG. 2. (a) Liver and (b) muscle glycogen content of yellow perch from naturally acidic Wharton Lake (ALP) and circumneutral Allequash and Trout lakes (NLP) collected throughout 1985–86. Means and 1 SEM are plotted. Sample sizes are available in Table 1. Note that muscle glycogen is reported as a concentration and liver glycogen is reported as the total amount, standardized to body mass.

tical removal of a storage time effect. Hepatic and muscle water contents changed significantly in both populations with season. There was more water in liver in summer than in autumn or winter. Muscle water was significantly higher in March compared with other dates (Table 3).

Proportion of red muscle

The fraction of total muscle that was red muscle was not population or season dependent, although there was a trend for more red muscle in NLP than in ALP (Fig. 5a).

Hepatosomatic index

Season and lake origin had an interactive effect on the hepatosomatic index of yellow perch (Fig. 5b). For every date, size-adjusted liver mass was greater in NLP than in ALP. In general,

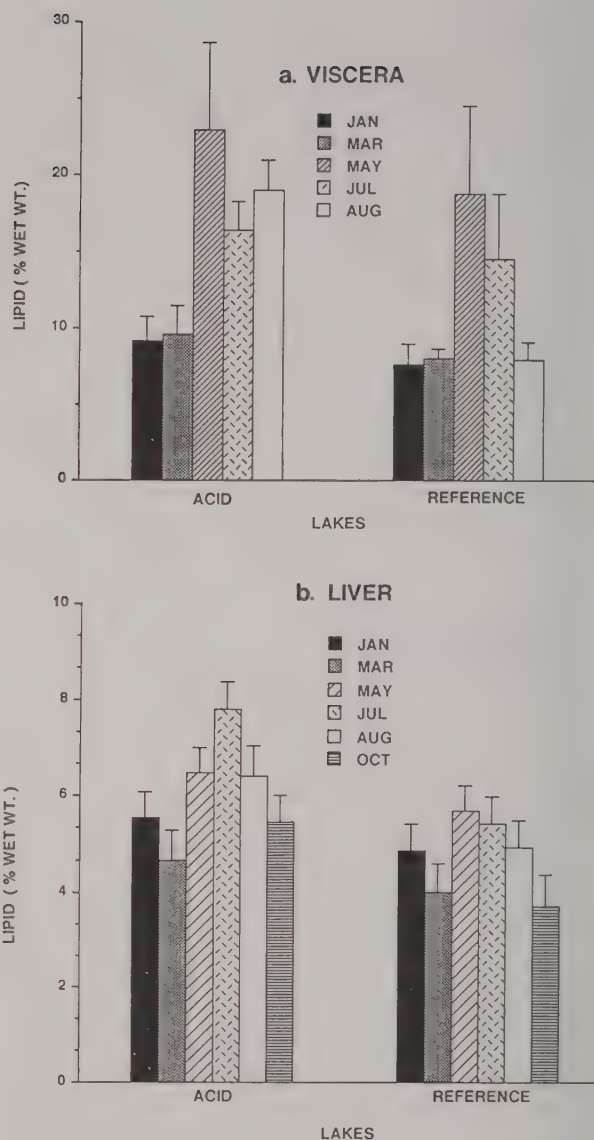


FIG. 3. (a) Visceral and (b) liver lipid content of perch. Details as in Fig. 2.

the trend for larger livers in NLP held for both sexes, but the lower values in July and October were the result of females having smaller livers.

Gonadosomatic index

Female ALP had a larger ratio of ovary size to body size than female NLP (Fig. 5c); teste size did not differ between populations. Season had a significant effect on the gonadosomatic index, since the gonads of both sexes proliferate throughout the winter months (Fig. 5c).

Discussion

Population Status

Little evidence supports the idea that adult ALP taken directly from their environment were physiologically stressed.

TABLE 3. Results summary for energy reserve and water content measurements not presented graphically. Mean values are presented with standard errors parenthetically underlain. Sample sizes are given in Table 1. All dates are for 1986 with the exception of the October collection which is from 1985. Ref = fish from the interconnected reference lakes Trout and Allequash, Wh = fish from naturally acidic Wharton Lake. — = not determined.

Response variable	Capture date											
	Jan. 2–4		Mar. 1–2		May 25–28		July 5–6		Aug. 8–10		Oct. 4–6	
	Ref	Wh	Ref	Wh	Ref	Wh	Ref	Wh	Ref	Wh	Ref	Wh
Lipid (% wet wt.)												
Muscle	1.53 (0.14)	1.56 (0.03)	1.31 (0.08)	1.45 (0.08)	3.09 (0.37)	3.15 (0.46)	2.46 (0.09)	2.73 (0.25)	2.17 (0.26)	2.42 (0.21)	1.79 (0.17)	1.67 (0.1)
Gonad	3.57 (0.19)	4.63 (0.29)	3.76 (0.21)	5.68 (1.9)	—	—	—	—	—	—	2.70 (0.31)	4.53 (0.52)
Gonad (♀)	4.08	4.98 (0.43)	3.96 (0.28)	3.89 (0.08)	—	—	—	—	—	—	2.90 (0.49)	4.53 (0.52)
Protein (% wet wt.)												
Muscle	8.78 (0.25)	8.38 (0.2)	10.1 (0.17)	9.86 (0.29)	—	—	13.7 (0.72)	11.2 (0.60)	16.1 (0.60)	16.4 (0.58)	8.71 (0.32)	8.02 (0.42)
Gonad	5.01 (0.62)	7.13 (0.58)	5.59 (0.25)	5.67 (0.36)	—	—	—	—	—	—	10.2 (1.5)	10.2 (1.5)
Gonad (♀)	9.26	8.26 (0.17)	6.09 (0.14)	6.14 (0.24)	—	—	—	—	—	—	12.2 (1.2)	10.2 (1.5)
Water content (%)												
Liver	72.3 (1.0)	74.3 (0.89)	73.7 (0.84)	76.6 (0.09)	76.3 (1.1)	77.3 (1.0)	78.9 (1.5)	76.7 (1.4)	79.2 (1.1)	76.0 (1.2)	73.9 (0.79)	72.7 (0.79)
Muscle	77.6 (0.27)	77.8 (0.18)	78.7 (0.25)	79.3 (0.24)	78.3 (0.31)	78.8 (0.26)	78.6 (0.24)	77.7 (0.37)	78.4 (1.0)	77.9 (0.19)	77.6 (0.24)	77.5 (0.20)
Gonad	75.7 (0.32)	76.5 (0.43)	79.8 (0.48)	80.1 (0.75)	—	—	—	—	—	—	78.4 (0.68)	74.6 (0.26)
Gonad (♀)	76.5	76.4 (0.50)	80.6 (0.40)	81.1 (0.58)	—	—	—	—	—	—	77.8 (0.40)	74.6 (0.26)

Differences in metabolite stores between ALP and NLP were usually opposite to most of the predictions from the literature on the effects of acid stress in fish. For example, Krishna Murthy et al. (1981), Lee et al. (1983), and Dheer et al. (1987) reported increased liver glycogen levels after chronic exposure of fish to acid in the laboratory, and Dheer et al. (1987) also reported decreased muscle glycogen levels. These results were opposite to the significantly higher muscle glycogen and lower liver glycogen levels we measured in ALP (Fig. 2). An insignificant trend for muscle protein to be lower in ALP (Table 3) was the only result consistent with predictions from the literature on acid stress (Bhaskar and Govindappa 1985).

The condition of ALP also suggested that they were not acid or nutrient stressed. ALP had significantly greater visceral lipid stores (the principal energy reserve in fish), and females allocated more of their available energy stores to reproduction (Table 2; Fig. 5c), while condition factor was similar in the two populations (Table 1). These results were somewhat surprising considering our qualitative observation of a greater density of perch in Wharton Lake than in the reference lakes.

Condition factor and enhanced reproductive effort of ALP were maintained at the expense of somatic growth (Fig. 1). Growth of fish populations exposed to cultural acidification is highly variable, depending primarily on trophic interactions and population density (Harvey 1982), and it seems reasonable to assume that this is true in naturally acidic systems as well. Although yellow perch respond to cultural acidification with increased growth before age 3 and reduced growth thereafter (Ryan and Harvey 1980), the slower growth in ALP after age 3 is as likely to have originated from density or year-class strength differences as from acidity per se and should not be

automatically attributed to acid-base or ion regulation costs. Keep in mind that the fish assemblages are more diverse in the neutral lakes than in Wharton Lake. Although NLP may have been present in lower densities because of piscivorous fishes, they might also have had diffuse competition for food resources from other fish species, and certainly their diet differed from that of ALP.

Population level differences in stored metabolites (present study), acid tolerance and total body sodium (Rahel 1983), blood chemistry (Nelson et al. 1988; Nelson and Mitchell 1992), and responses to exercise and recovery in acid water (Nelson 1989, 1990) suggest that ALP were not preadapted to the dystrophic environment. Implicit in this statement is the assumption that differences between the populations resulted from acclimatory or selective processes operating on a water chemistry gradient. Important confounding factors in this argument are interlake differences in piscine community structure and morphoedaphic factors. Wharton Lake historically lacked major piscivores (although largemouth bass (*Micropterus salmoides*) have been introduced several times since 1958 with poor success), while both reference lakes have healthy populations of fish that eat perch. In addition to the probable differences in predation pressure between populations, other differences (e.g. diet, density, habitat heterogeneity) between the lakes may have accounted for some of the interpopulation differences in metabolite levels.

We think that finding significant differences in stored metabolite levels between ALP and NLP in their natural environments supports our contention that ALP have compensated for the dystrophic environment (Nelson et al. 1988). Some of these differences are also consistent with

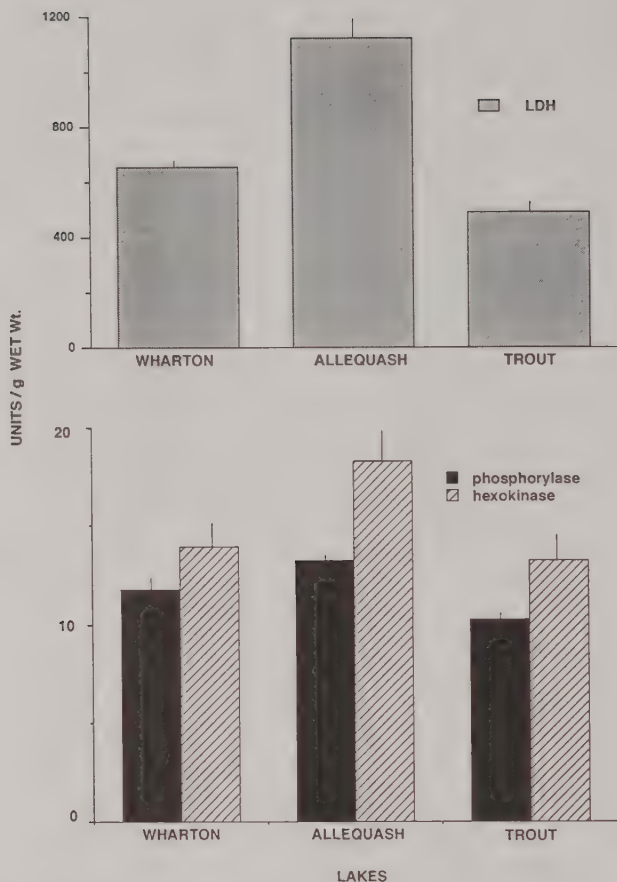


FIG. 4. Maximal activities of three enzymes of carbohydrate metabolism in yellow perch from acidic Wharton Lake (ALP) and circumneutral Allequash and Trout lakes (NLP) collected in May 1986. Means and 1 SEM are plotted. Sample sizes were 10 individuals for each lake.

speculative schemes for limiting endogenous acid production in ALP (see below). In addition to the population-level physiological differences between ALP and NLP (present study; Rahel 1983; Nelson et al. 1988; Nelson 1989, 1990; Nelson and Mitchell 1992) we also base our conclusion on analyses of fish community structure in temperate ecosystems that invariably isolate pH as an important factor in determining fish assemblages (cf. Rahel 1984; Tonn et al. 1990).

Limiting Endogenous H^+

Fish in acid water must excrete endogenously produced protons against a steeper environmental gradient than fish in neutral waters and may face additional acid diffusion from the environment (McWilliams 1982). It is reasonable to assume that the high energetic costs for acid excretion have been selected against over approximately 2500 generations since the lakes were formed. Of the potential mechanisms for limiting acid accumulation (i.e. swimming less, reduced proton permeability, increased buffering capacity), several are germane to the measurements reported here: (1) maximizing the glycolytic ATP/H^+ production ratio when using anaerobic metabolism (Hochachka and Mommsen 1983), (2) using relatively more aerobic metabolism to avoid anaerobic production of protons (Pörtner 1987), and (3) behaviorally avoiding extensive use of anaerobic metabolism.

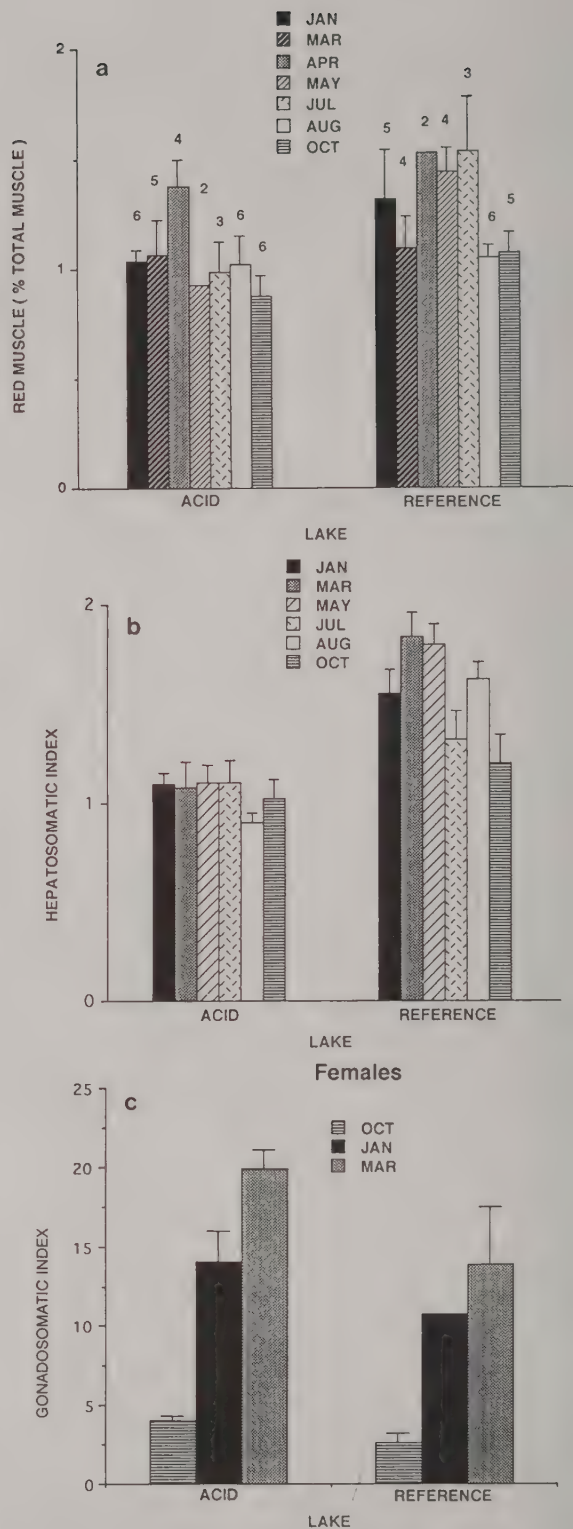


FIG. 5. Assorted morphometrics in perch from naturally acidic Wharton Lake and circumneutral Allequash and Trout lakes. (a) Red muscle content of yellow perch. Details as in Fig. 2. Sample sizes are posted on top of the error bars. (b) Hepatosomatic index (liver mass/body mass $\times 100$) of perch. Details as in Fig. 2. (c) Gonadosomatic index (gonad mass/body $\times 100$) of female perch throughout the winter of 1985–86. Details as in Fig. 2.

Hochachka and Mommsen (1983) have proposed on theoretical grounds that animals can maximize the ATP/H⁺ produced anaerobically by fermenting endogenous glycogen in muscle tissue instead of using glycogen from a central storage area (liver in fish). The acid-base benefits of this strategy are reversed during periods of muscle glycogen synthesis, but if ALP had been selected for minimizing acid production during exercise, we could expect them to rely more on muscle glycogen and less on liver glycogen. Comparison of stored glycogen levels supports this hypothesis (Fig. 2). ALP had smaller livers and less liver glycogen than NLP, and ALP usually had more muscle glycogen.

Storage levels of a metabolite are, at best, an approximation of its use by an animal and are subject to vagaries such as nutritional status and recent exercise history. Therefore, we examined maximal activities of two enzymes of glycogen metabolism in white muscle to further evaluate this hypothesis. Although in vitro maximal enzyme activities may not reflect actual in vitro reaction rates, GP and HK activities (Fig. 4) did not support the hypothesis that ALP are geared for preferential use of endogenous muscle glycogen. LDH activity was also measured to control for differences in anaerobic potential among the populations, and maximal activity of this enzyme varied significantly among perch from the three lakes in a pattern mirroring the GP and HK results (Fig. 4). Our interpretation is that the activities of these white muscle enzymes are varying in concert and that their levels are not influenced by environmental pH.

We also hypothesized that pressures to limit endogenous H⁺ production in ALP may have caused an increased ability to function aerobically in this population. Although a precise accounting of H⁺ production in anaerobiosis remains controversial (Hochachka and Mommsen 1983; Pörtner 1987), there is general agreement that avoiding anaerobic metabolism limits the metabolic acid load to the organism. Demonstration of a metabolic preference for fuels that can only be used aerobically would support the hypothesis that ALP derive a relatively greater amount of their energy aerobically. Enhanced aerobic function could arise by either increasing the fraction of muscle fibers poised for aerobic activity or increasing the aerobic capacity of existing glycolytic fibers. Red muscle cross-sectional areas were homogeneous between populations (Fig. 5a); therefore, it is unlikely that ALP are using the former strategy.

Differences in lipid stores supported the idea that ALP preferentially use aerobic metabolism. Unlike protein and carbohydrate, fish can only obtain energy from fatty acids (the chief component of stored lipids) aerobically, making them somewhat of a marker for aerobic metabolism. ALP had greater liver and visceral lipid content (Fig. 3); while muscle lipid content (mostly structural) was also higher in ALP, but not significantly (Table 3). Because of larger visceral and liver lipid stores and low hepatic glycogen levels, ALP probably rely on exogenous lipid and endogenous glycogen as energy sources for white muscle. Alternatively, ALP may behaviorally rely on red muscle and preferentially store fuels to supply that muscle. Because white muscle is recruited primarily at higher swimming speeds (Johnston 1982), this latter strategy is plausible only for populations subjected to low predation pressure. Interestingly, Nelson (1990) showed that ALP do not activate anaerobic glycolysis to the same extreme as NLP when motivated to swim to exhaustion.

Change in Life History Strategy

Differences in metabolite stores and growth rates between populations could also be a passive result of interpopulation

differences in life history strategy. A surprise of this study was finding that female ALP allocated more of their body stores to developing ovaries. Less reproductive investment has been reported for European perch in culturally acidified lakes (Valtonen and Laitinen 1988), suggesting again that acid stress is not responsible for our findings. Jansen (1990) found an identical increase in reproductive investment in a population of "stunted" perch from a central Alberta (Canada) lake, as well as a lower age at maturity in this population. This suggests that the higher gonadosomatic index (Fig. 5c) in ALP is the result of density-dependent changes in life history strategy. However, Jansen (1990) found lower visceral lipid stores in his "stunted" population, providing support for our idea that the greater lipid stores in ALP are the result of their environment, possibly the acidity in that environment.

Our conjecture is that directional selection has occurred for ALP and Jansen's (1990) "stunted" perch to invest more in reproduction because their commitment has been limited primarily by interactions between physiology, the environment, and nutrient availability, rather than species interactions. Owing to the absence of fish predators that eat perch in Wharton Lake, selection for antipredator adaptations could have been relatively low. This is supported by the relatively poorer swimming performance of ALP in neutral water (Nelson 1989). In contrast, NLP should have undergone stabilizing selection for ovarian investment; fish that sacrifice too much of their somatic stores to oogenesis are likely to increase their susceptibility to predation. This is again supported by swimming ability; gravid ALP had relatively poorer swimming performance than comparison animals whereas gravid NLP did not (Nelson 1989).

Conclusions

The results reported here provide further support for our contention (Nelson et al. 1988; Nelson 1989, 1990; Nelson and Mitchell 1992) that ALP have diverged physiologically from an adjacent "normal" perch population. We are unable to comment on whether these interpopulation differences in metabolite storage arose through H⁺-driven selection on extant genes, genetic drift, acclimatization, or life history changes unrelated to the physical environment. However, some of the interpopulation differences in metabolite storage were consistent with metabolic/behavioral adjustments that would limit endogenous H⁺ production and were at odds with expectations from population density differences alone. Our results also reject the notion that perch from naturally acidic lakes in northern Wisconsin are experiencing any of the physiological disturbances seen in fish from anthropogenically acidified waters.

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Water Flow and Clay Retention in Submerged Macrophyte Beds¹

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The effect of aboveground plant structure on near-bottom water flow and surficial sediment composition was evaluated in Lake Memphremagog (Quebec–Vermont) at 34 nearshore sites within three mixed-species macrophyte beds. Plant surface area accounted for 70% of the variance in flow reduction when the effect of changing water depth was removed. Relationships between plant surface area and surficial sediment clay content were evident within each of the three multispecies beds. The equations predicting within-bed clay concentrations were not significantly different from a general among-bed model ($r^2 = 0.74$) developed at 25 sites of high biomass from other nearshore locations in Lake Memphremagog. The study provides quantitative evidence for the importance of macrophytes as sites of sedimentation of fine particles and their associated nutrients and contaminants.

L'effet des structures végétales hors-terre sur le débit d'eau près du fond et la composition des sédiments superficiels ont été évalués dans le lac Memphremagog (au Québec et au Vermont) et 34 endroits situés près du rivage sur trois lits de macrophytes d'espèces mélangées. La surface des plantes comptait pour 70 % de la variance de la réduction du débit, lorsque l'effet du changement de la profondeur est supprimé. Le rapport entre la surface des plantes et la teneur en argile sédimentaire superficiel était évident dans chacun des trois lits d'espèces multiples. Les équations prévisionnelles des concentrations d'argile pour le lit n'étaient pas significativement différentes d'un modèle général de comparaison des lits ($r^2 = 0,74$) élaboré en 25 sites de biomasse élevée en d'autres endroits situés près du rivage dans le lac Memphremagog. L'étude apporte une preuve quantitative de l'importance des macrophytes comme sites de sédimentation de particules fines et de leurs éléments nutritifs et contaminants associés.

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Nearshore water flow comprises several components, including longshore currents, wind-driven deep- and shallow-water waves, and the refraction and reflection of wave energy from the shoreline, that together are modified by the physical obstruction presented by macrophyte beds. Many lakes support extensive macrophyte beds. Therefore, lakewide exchanges of water and solutes, as well as sediments and their associated nutrients and toxins, cannot be evaluated without consideration of processes in the littoral zone. As the energy environment determines whether particles will settle or be resuspended, a flow reduction within macrophyte beds should enhance their ability to retain sediments and their associated nutrients and contaminants. A significant flow reduction would imply that macrophyte beds serve as sediment traps for materials carried from the land and/or the pelagic zone. While the littoral zone is generally viewed as a source of nutrients and organic matter for the pelagic zone (Pieczynska 1986), there is some evidence that the littoral also serves as a sink for substances obtained from both the open water and the drainage basin (Schroder 1988; Petticrew and Kalff 1991a).

The present study aims at quantifying the role of macrophyte structure on both flow and sediment retention within the lake littoral zone. It does this by linking flows to submersed macrophyte forms for mixed- or monospecies beds. This has not been done quantitatively before, although modification of water flow by plant structure has been noted in aquatic environments

(Kouwen et al. 1969; Madsen and Warncke 1983; Marti and Tanner 1988). A study reporting measurements of water flow at five positions within a *Myriophyllum heterophyllum* stand (Losee and Wetzel 1988) lends support to the inferences of several authors that a buffering of wave action results in reduced flows within macrophyte stands (Keddy 1982; Pieczynska 1986; Kautsky 1987).

In this study, we measured water flow at 34 sites in and around three macrophyte beds in Lake Memphremagog (Quebec–Vermont) and developed the first model able to describe the effect of macrophytes on flow above the sediment surface. A within-bed model predicting the proportion of clay in the surficial sediment from plant surface area was also developed and compared with a general among-bed model comprising data from 25 other sites.

Methods and Materials

Study Sites

All aspects of this study were conducted in Lake Memphremagog during late August and early September 1985 and 1986 when macrophytes stands were at the stage of maximum biomass. Three littoral locations were selected to include macrophyte beds that exhibited a broad range of both growth forms and biomass (detailed later). These three locations represented different littoral energy environments, as characterized by the effective fetch (Hakanson and Jansson 1983), shoreline orientation, and bottom slopes (Fig. 1; Table 1). While all sites received the predominant southwesterly wind, the effective fetch ranged from 1.24

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FIG. 1. Lake Memphremagog sample locations, indicating the range of littoral exposures selected. Numbered markers identify the three within-bed macrophyte stands; the other 14 markers represent the locations where 25 among-bed sites were sampled.

(site 1) to 4.32 km (site 3). At each location, echo soundings, preceding the period of sampling, indicated regular bottom topography with the shore side of the macrophyte beds positioned a minimum of 30 m from the shoreline.

Macrophytes

At each of the three beds selected for within-bed measurements, 12 sites were selected for sampling, although sample loss reduced this to 11 sites for beds 1 and 3. A stratified random sampling method was used to select the 12 sites within regions of high, medium, and/or low to zero biomass. One or

more sites of zero or low biomass outside each of the beds were used to characterize unimpeded or plant-free flow. At beds 1 and 3, these were on the lakeward side whereas at bed 2 the site of lowest biomass was positioned shoreward of the extensive macrophyte bed. The macrophyte beds at all locations were of mixed-species composition, although bed 2 had an extensive (90 m) monospecific band of *Myriophyllum spicatum* bordered by a less dense region of mixed growth. The lakeward and shoreward extent of each macrophyte bed was visually delineated using SCUBA.

Macrophyte height was measured both in situ to record the position of the top of the plant canopy in the water column and in the laboratory to determine maximum plant height. When the period of flow measurement had ended, a SCUBA diver cleared four 900-cm² quadrats of plant cover at each site. This material was taken to the laboratory, washed, roots removed, and spun to remove excess water. The aboveground biomass was then sorted by species, weighed, and measured for height. The surface area of plants was determined using a dye adhesion technique (Cattaneo and Carignan 1983) modified for larger samples. Four plant species representing different growth forms were used to develop equations to estimate plant surface area from wet weight measurements. *Vallisneria americana*, *Potamogeton praelongus*, *M. spicatum*, and *Heteranthera dubia* represented long, ribbonlike leaves, broad, nondissected leaves, bushy, clustered, featherlike leaves, and slender, scattered, threadlike leaves, respectively (Fasset 1960). Significant linear relationships ($r^2 > 0.97$, $p < 0.001$) between wet weight and millilitres of adhered dye were found for each growth form. These values were then used in an equation predicting plant surface area from millilitres of adhered dye ($r^2 = 0.91$, $p < 0.001$). Macrophyte data were tabulated as plant surface area (square metres) per unit area of lake bottom (square metres), a ratio that is also referred to as leaf area index (LAI).

Physical Environment

Local underwater bottom slopes were calculated from depth measurements along a transect perpendicular to the shore. Measures for fetch (the distance from shore to shore over which the prevailing wind blows) were taken from the Canadian Hydrographic Service charts for Lake Memphremagog (No. 1361). The effective fetch was estimated using the method of Hakanson and Jansson (1983), which weights the overwater distances within an arc of 82° from the site.

Using the historical record of wind speeds and directions for the general region, individual sample sites were restricted to positions and depths commonly located offshore from the surf and swash zone. The refraction zone, where orbital motion begins to contribute to the bottom water velocities, was sampled in some cases, as plant biomass can be found in this region. The majority of sites were selected in water depths that were expected to be characterized by deepwater wave conditions (i.e. offshore of the refraction zone).

Wind speed and direction were recorded at 10-min intervals at a 10-m meteorological tower located in the watershed. In addition, a portable 2.45-m tower with a calibrated cup anemometer and telltales made from strips of flagging tape was located on the shoreline for manual hourly recordings of wind speed and visual observation of wind direction. Sufficient data to allow a correlation between the shore station and the continuously recording meteorological tower were collected to permit an estimate of the total windrun during each experiment.

With the measured wind speeds and period of duration, along with the calculated effective fetch, the characteristics of the

TABLE 1. Data table for 25 among-bed sites in Lake Memphremagog and the ranges for variables at the three mixed-species macrophyte beds.

Site	LAI	Water depth (m)	Slope (%)	Effective fetch (km)	Wave transition depth ^a	% clay
1	2.34	3.70	7.3	2.03	2.22	1.73
2	5.56	4.40	0.6	2.86	1.40	15.82
3	8.79	2.00	4.0	1.24	1.57	6.93
4	13.00	2.10	3.0	0.65	1.19	23.09
5	3.42	2.00	10.0	1.01	2.92	6.25
6	1.58	3.20	5.2	4.32	6.01	2.44
7	4.86	2.45	1.1	1.91	5.18	4.18
8	7.69	5.20	13.6	2.02	2.15	9.45
9	11.08	3.20	17.0	0.45	0.92	12.33
10	10.69	3.50	12.0	1.46	1.82	13.79
11	13.26	3.20	2.7	1.74	1.60	16.47
12	3.29	1.20	1.3	2.46	3.33	3.03
13	12.46	2.20	3.1	1.75	2.73	16.11
14	10.65	2.40	5.0	1.02	1.36	17.30
15	8.80	3.00	7.7	1.02	1.36	15.90
16	10.96	2.80	8.0	1.02	1.36	15.78
17	1.95	1.30	0.3	2.46	3.33	2.99
18	1.39	1.35	0.4	2.46	3.33	3.30
19	0.82	1.23	1.3	2.46	3.33	4.71
20	17.22	2.80	2.0	1.74	1.60	19.41
21	9.28	2.30	3.1	1.75	2.73	13.52
22	8.96	2.20	1.2	1.75	2.73	13.04
23	7.22	2.60	1.9	2.17	3.48	6.63
24	10.84	2.50	1.0	1.74	1.60	12.43
25	10.30	2.60	4.0	1.24	1.57	7.14
Bed 1			8.0	1.24	1.15	
Min	7.00	2.30				4.17
Max	13.90	4.00				11.75
Bed 2			3.3	2.17	0.63	
Min	0.28	1.10				3.31
Max	7.78	2.60				8.71
Bed 3			4.8	4.32	2.10	
Min	6.06	1.90				2.09
Max	14.11	3.50				11.22

^aCalculated using seasonal maximum (sites 1–25) or experimental maximum windspeeds (beds 1–3).

generated waves were calculated (Smith and Sinclair 1972). The wave transition depth (WTD) or the depth at which waves change from deep to shallow water waves was calculated from the wind record for the period of the experiments. This was done using both average windspeeds and maximum windspeeds, defined here not as gusts but as the highest windspeed maintained over a period of 3 h. This ensures appropriate time for the generation of waves, as predicted in the equations outlined by Bretschneider (1969).

Water flow at a position 25–30 cm above the sediment–water interface was measured by gypsum cylinder dissolution. Weight loss per unit area of gypsum cylinder was used to estimate the mean flow for the 51-, 29-, and 47-h experiments. Water temperatures for the period the gypsum was deployed were measured, as gypsum dissolution is a function of both flow and temperature (James and Lupton 1978). Field calibration of the gypsum cylinders in this same water body resulted in standard deviations for flow estimates between 0.12 and 0.20 cm·s⁻¹ ($n = 4$ and 16, respectively) (Petticrew and Kalff 1991b). The field calibrations exhibited mean flows in a range similar to those measured during the macrophyte bed sampling. At each site, divers carefully placed (and at the end of the experiment

removed) four preweighed replicate gypsum cylinders approximately 25 cm above the sediment–water interface. In the laboratory, visual inspection for cylinder chipping or the presence of biofilms was done. Chipping resulted in the loss of several replicate cylinders, but no biofilms were noted from exposure periods of up to 51 h. Final weights of oven-dried samples were used to calculate weight loss per unit area, from which the flow was estimated using the equations presented in Petticrew and Kalff (1991b).

To account for the effect of water depth on changing bottom water flows, a plant-free nearshore area with identical topography was assumed and the unimpeded depth-dependent flows were calculated. This was done by weighting the values of measured flow at the site of zero (or lowest) biomass, by the relative differences in water depth between it and all other sites:

$$(1) F_I = (D_I/D_O) \cdot F_O$$

where F_I = water flow estimate for a site inside the macrophyte bed, F_O = water flow measured at a site outside the macrophyte bed, D_O = depth of water above the gypsum cylinder outside the bed, and D_I = depth of water above the gypsum cylinder inside the bed. Assumptions incorporated by this model are discussed later.

Horizontal flow associated with orbital motion was calculated from equations presented in Pond and Picard (1978). When orbital motion, as calculated from either the average or maximum sustained windspeed, contributed significantly (i.e. $>0.10 \text{ cm}\cdot\text{s}^{-1}$) to bottom flow at the water depths where the gypsum cylinders were positioned, the calculated unimpeded flow was considered to be the sum of the orbital component and depth-dependent flow.

Sediment Sampling

SCUBA divers used 6.5-cm-diameter handheld cores to sample the bottom sediments to a depth of ~ 20 cm. Triplicate samples were taken at each site. Approximately 2 g of wet sediment was collected by scraping the top 3 mm from one of the three replicate cores. This material was stored at 4°C and the particle size structure between 0.63 and $645 \mu\text{m}$ was later analysed using a Coulter counter technique (Kranck and Milligan 1979). This allowed for the calculation of the clay content which includes sediment smaller than $2 \mu\text{m}$. The organic content of this surficial material was determined by wet ashing with hydrogen peroxide (Lavcolich 1975). The top 1 cm of the other two cores from each site was extruded and over-dried at 60°C to determine water content.

Among-Bed Comparison Sites

Twenty-five sites at 14 other shore locations in Lake Memphremagog were sampled for surficial sediment and plant biomass (Fig. 1). The sites of high biomass along these transects, which represent a wide range of shoreline exposures and macrophyte bed compositions, were used to evaluate the among-bed relationships found between plant structure and surficial sediment composition. The physical characteristics of these sites (water depth, fetch, bottom slope) and the range of above-ground plant biomass sampled are given in Table 1.

Results

Flow modification was calculated as the numerical difference between the measured mean flow, as determined from gypsum flux, and the calculated unimpeded mean flow. Only at bed 3 was the contribution from orbital flow found to be significant. Strong onshore winds were experienced for 7.5 h during this 47-h experiment. Horizontal flows of $1 \text{ cm}\cdot\text{s}^{-1}$ at water depths of 1.6 m were calculated. Therefore, the unimpeded mean flow at bed 3 was considered the sum of depth-dependent flow and a time-weighted portion of the contribution from orbital flow, as calculated from the maximum sustained windspeed. At the other two beds, windspeeds and directions were more consistent; the generated waves did not contribute significantly to bottom water velocity at the sample sites. Therefore, mean unimpeded flow is represented by the results of equation 1 at these two locations.

Values of flow modification were combined for all experiments and plotted against LAI (Fig. 2). Negative values of flow modification mean that the measured flows were less than the flows calculated for the hypothetically plant-free area. For the complete data set ($n = 34$), 70% of the variance in flow modification is explained by LAI, with a decrease in flow as LAI increases. When the beds are analysed separately, each exhibits a significant ($p < 0.05$) relationship between flow modification and LAI and their slopes and intercepts are not significantly different from each other (Table 2).

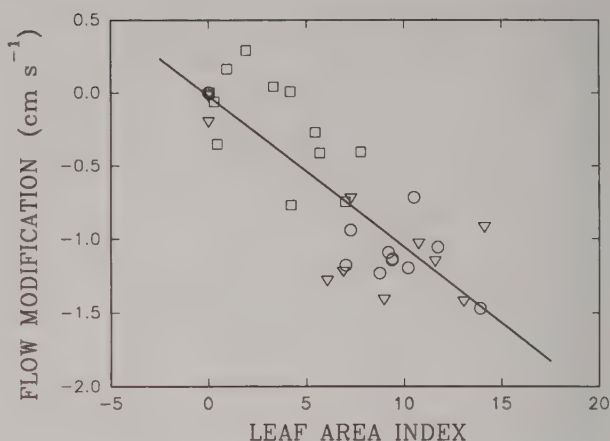


FIG. 2. General relationship between LAI and flow modification for the combined data set. Flow modification = $-0.103 \times \text{LAI} - 0.024$ ($r^2 = 0.70$, $n = 34$). Bed 1 is represented by circles, bed 2 by squares, and bed 3 by triangles. Individual bed relationships are all statistically similar to the combined model and are presented in Table 2.

TABLE 2. Comparison of the combined mixed-species model ($n = 34$) and the three individual bed models. Each model has the form flow modification ($\text{cm}\cdot\text{s}^{-1}$) = $m \times \text{leaf area index} + b$. Slopes and intercepts are presented with their standard errors below. $**p < 0.01$, $*p < 0.05$.

Model	r^2	n	Slope	Intercept
Combined	0.70**	34	-0.103 0.012	-0.024 0.306
Bed 1	0.68**	11	-0.090 0.021	-0.218 0.229
Bed 2	0.36*	12	-0.076 0.032	0.050 0.285
Bed 3	0.65**	11	-0.084 0.020	-0.260 0.336

The proportion of clay ($<2 \mu\text{m}$) in the surficial sediments within the beds increased as LAI increased. Individually the three locations show similar log-linear relationships, although bed 3 exhibits a larger scatter and a poorer fit (Fig. 3; Table 3). The 25 among-bed comparison sites show that 74% of the variance in the log (% clay) is explained by LAI. The slope and intercept of this among-bed relationship are not significantly different from those of beds 1 and 2, indicating that the models developed within single macrophyte beds are similar to those developed from among a range of littoral sites (Table 3) and are therefore not bed specific.

Discussion

Water flow in the lake littoral is a mixture of several components of nearshore flow. Our goal was not to identify and quantify each component but rather to determine and model the relationship between plant structure and time-averaged water flow just above the sediment surface, as it is there that flow has its largest effect on sediment resuspension. It is evident that LAI has a quantifiable dampening effect on flow within macrophyte beds (Fig. 2). The general, combined model describing this is based on 34 sites from three macrophyte beds and incor-

TABLE 3. Statistics for the individual macrophyte bed models and the among-bed comparison model developed from 25 sites at 14 other locations in Lake Memphremagog. The unadjusted model for the six sites exhibiting high *M. spicatum* biomass is also presented. The form of each model is $\log (\% \text{ clay}) = m \times \text{leaf area index} + b$. Slopes and intercepts are presented with their standard errors below. ** $p < 0.01$, * $p < 0.05$, NS = not significant.

Model	r^2	n	Slope	Intercept
Bed 1	0.67**	10	0.054 0.013	0.339 0.081
Bed 2	0.81**	11	0.057 0.009	0.472 0.077
Bed 3 (includes outliers)	0.004NS	8	-0.005 0.033	0.867 0.261
Bed 3	0.45NS	6	0.023 0.013	0.716 0.089
Unadjusted <i>M. spicatum</i> sites	0.86**	6	0.014 0.003	0.674 0.032
Among-bed sites	0.74**	25	0.063 0.008	0.434 0.167
Combined model	0.69**	52	0.053 0.005	0.465 0.147

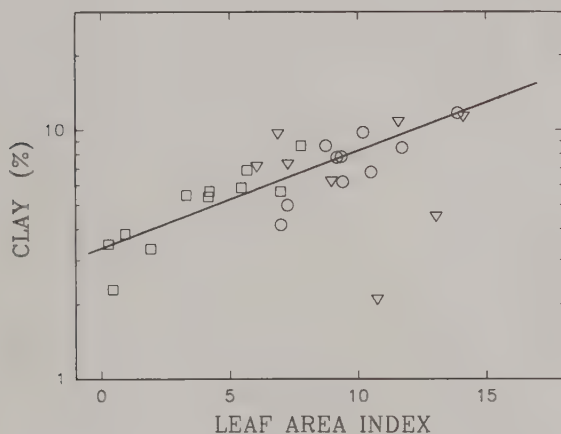


FIG. 3. Relationship between LAI and $\log (\% \text{ clay})$ for the three within-bed locations. Excluding the two outliers (bottom right), the line of best fit is $\log (\% \text{ clay}) = 0.039 \times \text{LAI} + 0.526$ ($r^2 = 0.73$, $n = 27$). The two outliers from bed 3 are discussed in the text. Bed 1 is represented by circles, bed 2 by squares, and bed 3 by triangles.

porates a large biomass range ($0\text{--}3.35 \text{ kg wet weight} \cdot \text{m}^{-2}$) of mixed-species assemblages. The three location-specific models are not statistically different from each other, indicating that while the beds experienced different energy regimes and exhibit different characteristics (local bottom slope, exposure, bed dimensions), the effect of plant structure on flow modification is similar. This then allows for the use of the combined model to predict flow modification in a wide range of macrophyte beds.

The assumptions involved in the use of equation 1 to estimate unimpeded depth-dependent flow require first that bottom slopes in the area of measurement are insignificant in the cross-shore direction in comparison with the onshore, such that water flow can be considered two-dimensional, changing only as a function of depth. Echo sounding and mapping of the bottom

topography of the sample locations show that this requirement is fulfilled. The second assumption is that in the region of flow measurement, bottom roughness, as determined by bedforms and/or sediment texture, does not vary significantly. This constraint implies that the vertical structure of the velocity profiles is similar at all onshore locations, with the shear velocity (u_* , which varies only as a function of depth) being the single relevant parameter. Analyses of surficial bottom sediments from the deep and shallow boundaries of these macrophyte beds show the maximum range to vary between 128 and 406 μm . This does not provide a sufficient difference in bottom roughness to invalidate the second assumption. Caution may be necessary if orbital motion from waves comprises a significant component (i.e. $>20\%$) of the flow for long periods. In bed 3, at several sites, orbital motion from wave action exceeded 20% of the mean flow, but this occurred for only 16% of the period of the experiment. For all the bed 3 sites the calculated orbital velocities were added to the predicted values of unimpeded depth-dependent flow. Some of the error in the bed 3 model may be a function of the relationship between these two velocities not being additive over all flow ranges.

Surface Area Adjustments

Six samples were noted to be outliers from the general relationship between flow modification and LAI within the sample set of 34 sites. These exhibited not only a very high *M. spicatum* biomass (Table 4), but also one that extended to the top of the water column where it branched out and in some cases grew horizontally. As flow was measured in the bottom 25 cm and as the distribution of the biomass throughout the water column is an important factor in regulating sediment movement, the biomass at sites of extensive *M. spicatum* had to be adjusted to account for this structural biomass asymmetry. Adams et al. (1974) reported that 58% of the biomass was found in the top portion of the water column at periods of maximum biomass when *M. spicatum* grows to the water surface. Using this observation, sites of tall (water depths exceeding 2.5 m and with laboratory-measured plant heights equal to or exceeding the depth of the water column), dense (biomass $>1.3 \text{ kg} \cdot \text{m}^{-2}$) *M. spicatum* were adjusted. In each of the six cases, 42% of the measured *M. spicatum* biomass was used to calculate surface area through the height of the water column. The six corrected *M. spicatum* sites exhibit a good fit with both the combined and the individual macrophyte bed models (Table 2).

The relationship between LAI and $\log (\% \text{ clay})$ also responded well to the *M. spicatum* biomass correction. Without the correction the six outlier samples form a separate, significant ($r^2 = 0.86$, $n = 6$, $p < 0.01$) linear relationship between LAI and $\log (\% \text{ clay})$, which has a slope significantly different from the other models (Table 3). This means that if $\log (\% \text{ clay})$ is to be predicted from LAI, either the separate relationship for high *M. spicatum* biomass can be used or the high biomass samples can be corrected for the biomass asymmetry and used in the general models.

Surficial Sediment Predictions

There are two outliers in the $\log (\% \text{ clay})$ – LAI relationship (Fig. 3 and 4). These are shallow-water sites from bed 3 that have a significant amount of plant biomass with relatively low clay concentrations in the surficial sediment. Table 1 shows that the wave transition depth for this experiment was 2.1 m, meaning that at shallower depths, waves underwent a transition from deep- to shallow-water types. As a result, wave energy at

TABLE 4. Values for the six sites in the experimental beds that were corrected for tall, dense *M. spicatum* biomass. At sites where *M. spicatum* reached to the top of the water column and where wet biomass exceeded $1.3 \text{ kg} \cdot \text{m}^{-2}$, 58% of the biomass was excluded from the LAI calculations (see text).

Bed	Total wet biomass ($\text{kg} \cdot \text{m}^{-2}$)	<i>M. spicatum</i> biomass ($\text{kg} \cdot \text{m}^{-2}$)	Water depth (m)	Initial LAI	Corrected LAI	Flow modification ($\text{cm} \cdot \text{s}^{-1}$)
1	2.39	1.73	3.1	18.67	10.19	-1.20
1	2.02	1.27	2.8	15.39	9.18	-1.09
1	2.17	1.54	3.0	16.91	9.36	-1.14
2	2.19	2.19	2.6	18.53	7.78	-0.41
2	1.59	1.59	2.5	13.43	5.68	-0.41
3	3.35	2.82	2.7	27.97	14.11	-0.92

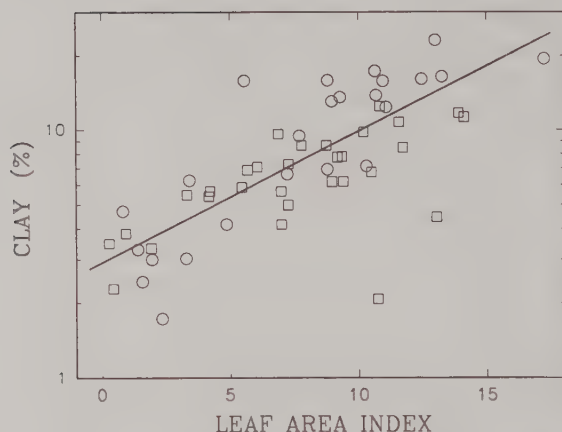


FIG. 4. Combined data sets of within- and among-macrophyte bed samples. Two shallow-water outliers have been excluded from the statistical analysis (see text) resulting in the general relationship $\log (\% \text{ clay}) = 0.053 \times \text{LAI} + 0.465$ ($r^2 = 0.69$, $n = 52$). Within-bed samples are represented by squares; among-bed sites are represented by circles.

these two shallow sites was sufficient to resuspend and export fine particles whereas at the deeper sites the bottom sediments would not be affected by the wind-generated waves. The linear fits for the $\log (\% \text{ clay}) - \text{LAI}$ relationships have therefore excluded the two outliers which represent a different energy environment than the remaining 32 sites. (In Table 3 the fits, both excluding and including the two outliers, are presented for bed 3.) The wave transition depths for approximately half of the 25 comparison sites are greater than the sampling depths (Table 1). However, for these sites the wave transition depth was calculated from the maximum sustained 3-h windspeed for the full summer season and therefore does not necessarily reflect the sedimentation environment directly preceding the late-summer sampling of these 25 sites. Variation in the sediment models may also be related to the degree of cohesiveness the surface sediments exhibit. As sediment cohesiveness increases, greater amounts of energy are required for resuspension. This characteristic of the sediment was not measured in the field and may explain some of the model and/or outlier error.

Models allowing the prediction of surficial sediment composition in the littoral have not previously incorporated characteristics of macrophyte beds, although their effect has been postulated (Carpenter 1981; Hakanson and Jansson 1983). The models presented (Fig. 3 and 4) show that at such sites, plant structure above the sediment-water interface is able to explain 69% of the variance in clay content. Other work (Petticrew and

Kalff 1991a) shows that the littoral surficial sediment structure, of which clay forms a part, is predictable from variables including water depth, effective fetch, bottom slope, sediment organic matter, and a dummy variable representing the presence or absence of plants.

Macrophytes play a crucial role in providing a suitable physical environment for the sedimentation of fine particles and their associated nutrient, heavy metal, and organic contaminants in the littoral zones of lakes, a sedimentation that has hitherto been thought to occur only at deep profundal sites (Lehman 1975; Hilton 1985). Limnologists have normally considered the littoral as a source of materials to the pelagic zone rather than a sink (James and Barko 1991). However, macrophytes allow the littoral to serve both as a seasonal and a long-term sink of materials. Work on Lake Constance shows the beds to have served as sites of net sediment accumulation over hundreds if not thousands of years (Schroder 1988). The removal of macrophytes, as the result of increased eutrophication, led to a rapid loss of the accumulated sediments. The extent to which the littoral zone serves as a short-term and long-term sink of nutrients and contaminants is not yet predictable but is at least partly a function of both the density of the beds and their seasonal stability (i.e. the extent to which they overwinter intact and the extent to which they are protected from summer storms (L. Jackson and J. Kalff, in prep.)). It is evident that the littoral and pelagic zones are closely linked and that good predictions about events in one will require an equal appreciation of the other.

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Estimating the Mud Deposition Boundary Depth in Lakes from Wave Theory¹

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Rowan, D. J., J. Kalff, and J. B. Rasmussen. 1992. Estimating the mud deposition boundary depth in lakes from wave theory. *Can. J. Fish. Aquat. Sci.* 49: 2490–2497.

The mud deposition boundary depth (mud DBD) is the depth in lakes at which the boundary occurs between high-energy erosive environments (coarse-grained noncohesive sediments) and low-energy depositional zones where fine-grained cohesive sediments accumulate. We have derived a model from the theory of waves and sediment thresholds that predicts the upper limit to the distribution of fine-grained sediments in lakes of any size. Our results suggest that the several biggest storms each year, rather than extremely rare events, are responsible for the upper limit to the distribution of mud. However, significant areas of coarse-grained sediments and many mud DBDs occur deeper than this upper limit, usually on slopes greater than 3%. For sediment at the mud DBD (23 μm), we have developed an empirical relationship between slope and maximum horizontal velocity that demonstrates the significant effect of slope on reducing either sediment threshold velocity or sediment stability.

La profondeur de démarcation des dépôts (PDD) de boue est la profondeur des lacs à laquelle se situe la démarcation entre les milieux érosifs à énergie (sédiments non cohésifs grossiers) et les zones de dépôt à faible énergie où les sédiments cohésifs fins s'accumulent. Nous avons dérivé un modèle de la théorie des vagues et des seuils de sédimentation qui permettent de prévoir la limite supérieure de la distribution des sédiments fins dans les lacs de toutes dimensions. Nos résultats indiquent que plusieurs gros orages par an, et non des événements extrêmement rares, déterminent la limite supérieure de la répartition de la boue. Cependant, des zones importantes de sédiments grossiers et de nombreuses PDD se trouvent à des niveaux plus profonds que cette limite supérieure, habituellement sur des pentes de plus de 3 %. Pour les sédiments à la PDD de boue (23 μm), nous avons élaboré une relation empirique entre la pente et la vitesse horizontale maximum qui démontre l'effet important de la pente sur la réduction de la vitesse du seuil de sédimentation ou de la stabilité des sédiments.

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Laboratory studies demonstrate the dependence of sediment threshold velocity on particle size for unconsolidated, noncohesive sediments (Komar and Miller 1975; Miller et al. 1977), but in situ comparisons of particle size and wave velocity during quiescent summer periods typically find coarser sediment than expected, suggesting that sediment distribution is either a function of severe winter events (Komar et al. 1972; Drake et al. 1985) or that laboratory results are not directly applicable to field situations. In lakes, when direct measurements of waves are lacking, estimates of wave energy as a function of wind and fetch also produce underestimates of particle threshold size (Sly and Sandilands 1988). If sediment distribution is controlled by severe events, then either theoretical or empirical approaches that can account for extreme wave conditions are necessary to accurately describe sediment distribution in lakes. Indeed, empirical approaches relating depth and fetch to sediment distribution in several lakes suggest that sediment distribution, even if controlled by extreme events, can be estimated with simple empirical models (Håkanson 1977, 1981). The link between wave energy and sediment distribution is likely to be more direct in lakes than in marine systems, where tides and currents can have important modifying effects, but may be confounded by the effects of slope, which can lead

to coarser-grained sediments than expected from wave energy alone (Håkanson 1977, 1981).

In this paper, we first identify the mud deposition boundary depth (mud DBD) as the depth of the abrupt transition from coarse-grained noncohesive sediments of high-energy erosional environments to fine-grained cohesive sediments of low-energy depositional environments. We then provide a multilake test of wave and particle threshold theory as predictors of the mud DBD by simplifying the theory to equations requiring only site depth and the wave height surrogates maximum fetch or exposure. For each site, we calculate and compare maximum horizontal velocity at the bottom with the particle threshold velocity for sediments at the mud DBD. In this manner, we classify sites as high energy or low energy and then compare these environmental energy classifications with classifications based on sediment texture (coarse-grained or fine-grained). Initially we assume maximum wave conditions and then use the observed sediment distribution to estimate the wave height that provides the best prediction of sediment distribution. Finally, we show that most of the discrepancies between theoretical and observed sediment distribution can be explained by slope effects, and we present theoretical–empirical and empirical models that account for these effects.

Methods

The sampling protocol used for this study consisted of first examining a bathymetric map of the particular lake to be sam-

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TABLE 1. Comparison of lake morphometry and sample sites.

Lake	Surface area (km ²)	Maximum depth (m)	No. of sample sites	Sediment type	
				Coarse (no.)	Fine (no.)
Superior, Canada–USA (Cook 1975)	82 367	406	379	116	263
Huron, Canada–USA (Thomas et al. 1973)	41 475	245	169	84	85
Georgian Bay, Canada (Loveridge and Cook 1976)	15 111	165	108	44	64
North Channel, Canada (Loveridge and Cook 1976)	3 950	85	53	24	29
Vänern, Sweden (Håkanson 1975)	5 893	106	113	19	94
Vättern, Sweden (Håkanson and Ahl 1976)	1 856	128	94	42	52
Memphremagog, Canada	90	117	199	103	96
47 lakes, Canada	10.05–31	2–86	145	45	100
			1260	477	783

pled and identifying the orientation of a transect (or transects) that expressed the range in slopes and depth for that basin. Then at the lake, high-resolution echo-sounder transects were recorded and subsequently examined for sampling site selection. The site selection process considered both depth and slope, with sampling proceeding from near shore to maximum depth, so that the range in sediment types present in the basin were characterized. In total, we sampled 344 sites in Lake Memphremagog and 47 other Quebec and Ontario lakes (Table 1). Surficial sediment (0–2 cm) water content was determined by drying (60°C) samples from 5 to 10 cores or Ekman dredges at each site.

These data were augmented with published data for surficial sediment (0–3 cm) mean particle size (ϕ) from Lakes Superior (Cook 1975) and Huron (Thomas et al. 1973), Georgian Bay (Loveridge and Cook 1976), and North Channel (Loveridge and Cook 1976) and surficial sediment (0–1 cm) water content from Lakes Vänern (Håkanson 1975) and Vättern (Håkanson and Ahl 1976 (Table 1).

At sites sampled for this study, depth (metres) was obtained from high-resolution echo-sounder records. For published data, site depth was given. The wave height surrogates (maximum fetch and exposure) were determined from maps. Maximum fetch (kilometres) is the maximum straight-line distance to shore from the sample site. Exposure (square kilometres) is the circular integral of fetch (Rasmussen 1988a) and describes the area of water visible from a site. Slope (percent) was determined from the high-resolution echo-sounder transects oriented perpendicular to contours at sites sampled for this study and from bathymetric maps for published sites.

Statistical Methods

The sites were classified into two groups based on sediment texture: coarse-grained and fine-grained. We used a variety of nonlinear regression techniques (Wilkinson 1989) to fit lines that best separate the two groups of sites, or to evaluate buried coefficients and exponents. First, we used a nonlinear iterative technique to empirically define an upper limit to the distribution of fine-grained sediments, while optimizing the total number of correct classifications (both fine-grained and coarse-grained), using the variables depth and either maximum fetch or exposure. Next, we used nonlinear regression (Wilkinson 1989) to

evaluate the buried exponents and coefficients for our wave height surrogates (maximum fetch and exposure) that correspond to the empirical fits. This enabled us to derive wave height, maximum horizontal velocity, and particle threshold velocity equations in terms of these surrogates that also reflect the empirical results. Then, we again used a nonlinear iterative regression to fit a line that best separates fine-grained and coarse-grained sites that were at depths greater than the previously defined upper limit, using slope and theoretical estimates of maximum horizontal velocity. In this manner, we obtained a theoretical–empirical estimate of an apparent threshold velocity. Finally, in a totally empirical approach, we used nonlinear logistic regression (Wilkinson 1989) to best separate the two groups of sites using the variables depth, slope, and either maximum fetch or exposure.

The results of the nonlinear regression analyses do not lend well to graphical presentation, so to graphically present our results, we identified 254 mud DBDs in our data. These observed mud DBDs are plotted against the predictions of the nonlinear regression analyses but were not used to generate any of the statistical results. For our study sites, mud DBDs were identified between adjacent coarse-grained and fine-grained sites using high-resolution echo-sounder records. For literature sites, mud DBDs were identified at the midpoint depth between adjacent coarse-grained and fine-grained sites that differ in depth by less than 10%.

To demonstrate the equivalence of site minima at 60% water content and 5.5 ϕ , we used the data of Cline and Chambers (1977) from Sleeping Bear Point, Lake Michigan. Cline and Chambers (1977) did not report ϕ , but did report both water content and sand–silt–clay percentages. We converted their sand–silt–clay percentages to ϕ with a regression we derived from the Lake Huron sediment data of Thomas et al. (1973):

$$\phi = -3.647 + 0.053 \text{ sand} + 0.086 \text{ silt} + 0.138 \text{ clay} \quad r^2 = 0.98, \text{SE}_{\text{est}} = 0.35, n = 169.$$

Results

Mud Deposition Boundary Depth (Mud DBD)

Bimodality of particle sizes within sediment samples has been noted for many aquatic environments (Folk and Ward 1957; Spencer 1963; Thomas and Kemp 1972; Pettijohn 1975). Modes

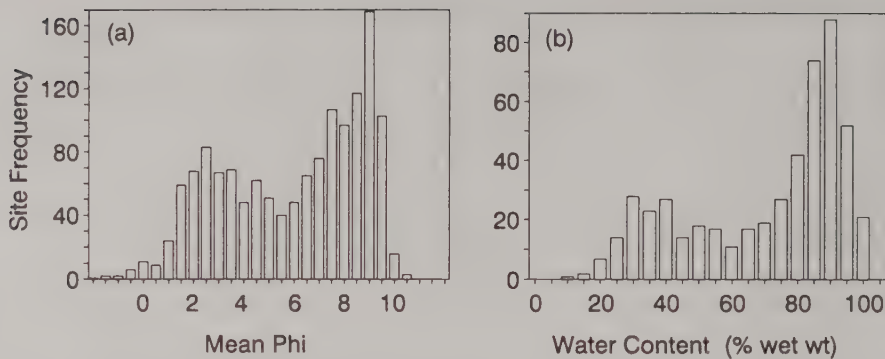


FIG. 1. Site frequencies of (a) mean particle size (ϕ) and (b) water content. Site frequencies are bimodal with intermodes at 5.5 ϕ and 60% water content. Site frequency of ϕ from published data for 1043 sites in Lakes Superior and Huron, Georgian Bay, North Channel (Table 1), and Lakes Michigan (Cahill 1981), Erie (Thomas et al. 1976), and Ontario (Thomas and Kemp 1972), water content from published data from Lakes Vänern and Vättern (Table 1), and original data from Lake Memphremagog and 47 other lakes in Quebec and Ontario.

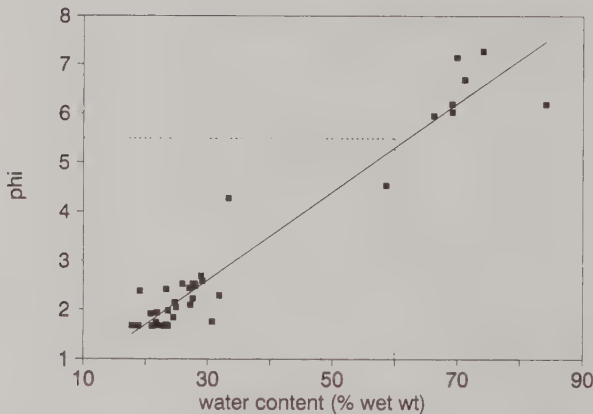


FIG. 2. Plot of ϕ versus water content for the sediments of Sleeping Bear Point, Lake Michigan (data from Cline and Chambers 1977), demonstrating the equivalence of 5.5 ϕ and 60% water content. $\phi = -0.093 + 0.090 \text{ water content}$, $r^2 = 0.93$, $SE_{\text{est}} = 0.47$, $n = 39$.

occur in gravel (-3.5 to -2 ϕ), sand (1 to 4 ϕ), and clay (7 to 10 ϕ), with intermodes in fine gravel (-1 to 1 ϕ) and coarse silt (5 to 6 ϕ). The within-sample pattern is, of course, repeated between sites as is evident on a frequency plot of mean particle sizes for sites in the Laurentian Great Lakes (Fig. 1a). Modes occur at 2.5 ϕ (sand) and 9 ϕ (clay), with a minimum of sites with a mean particle size of 5.5 ϕ . The gravel mode is absent in the Laurentian Great Lakes. An equivalent bimodality in site frequency is observed for sediment water content (intermode at 60%) (Fig. 1b), an important physical property of sediment (Fukada and Lick 1980; McCave 1984) related to particle size (Håkanson 1977) and bulk density (Richards et al. 1974). Modes in site frequency occur at 30% (sand) and 90% (clay) water content with a minimum of sites at 60% water content (Fig. 1b).

The equivalence of the site minima at 5.5 ϕ and 60% water content can be demonstrated with data from Sleeping Bear Point, Lake Michigan (Cline and Chambers 1977). A regression of ϕ versus water content for the Lake Michigan data yields the following result (Fig. 2):

$$\phi = -0.093 + 0.090 \text{ water content} \\ r^2 = 0.93, SE_{\text{est}} = 0.47, n = 39.$$

Thus, a mean particle size of 5.5 ϕ corresponds to a water content of 62.2%, confirming the equivalence of the site minima.

The site minima at 5.5 ϕ (23 μm) and 60% water content observed at many lakes are nearly coincident with the laboratory-observed transition from noncohesive to cohesive behaviour for quartz at a particle size of 10 μm (McCave 1984). Although sediments in lakes are mixtures of particles often affected by biological activity and may exhibit some cohesive behaviour at particle sizes greater than 10 μm , the site minima at 23 μm (5.5 ϕ) most likely represent the transition between the very different physical behaviour of noncohesive and cohesive sediments. In addition, the site minimum at 5.5 ϕ (23 μm) is generally coincident with the transition from high-energy erosive environments to low-energy depositional environments (Stanley et al. 1983). Another important feature of this transition is its abruptness. In the 48 lakes sampled for this study, the transition is very abrupt, usually occurring over a horizontal distance of a few metres.

Thus, the site minima are coincident with the abrupt transition of sediment texture (coarse-grained noncohesive to fine-grained cohesive), environmental energy (high to low), and sedimentary process (erosional to depositional). We define the depth of the abrupt transition between these distinct sedimentary environments at 5.5 ϕ (23 μm) or 60% water content (Fig. 1 and 2) as the mud DBD. The mud DBD is essentially equivalent to similar concepts such as the mudline of continental margins (Stanley et al. 1983) or the zone of transportation in lakes (Håkanson 1977, 1981).

If waves are solely responsible for the distribution of sediments in lakes, then the mud DBD should occur where maximum horizontal velocity (U_m) equals threshold velocity for 23 μm particles (U_{t23}). However, Håkanson (1977, 1981) has shown that slope can have an important modifying effect on the distribution of sediments in lakes. We hypothesize that slope effects can result in coarse-grained sediments (and mud DBDs) at depths greater than expected from waves alone. To differentiate the observed mud DBD, which may reflect slope as well as wave-particle interactions, from the theoretical predictions of wave and particle threshold theory, we define the

depth of the equivalence of U_m and $U_{1/23}$ as the mud energy boundary depth (mud EBD).

In sedimentary environments controlled by wave energy, mud should never be found at depths shallower than the mud EBD because wave energy above the mud EBD should be sufficient to transport and deposit sand. Environments where the distribution of sediments may be controlled by factors other than wave energy and where mud may be found above the mud EBD include (a) lakes with extremely high sediment supply which overwhelms wave energy (McCave 1971; Stanley et al. 1983), (b) extremely shallow lakes with long water residence times, where resuspension does not result in removal of fine particles by outflow, and (c) lakes with sedimentary environments dominated by the wave damping effects of aquatic macrophyte beds. Such lakes and sites will not be considered in this analysis. Thus, in our analysis the mud EBD should define an upper limit to the distribution of mud, but if slope has important effects, sand (and mud DBDs) may be found at depths below the mud EBD.

The relationship between particle size and wave energy at the mud EBD should generally be valid, with exceptions associated with out-croppings of fine-grained consolidated or semiconsolidated glacial-lacustrine deposits above the mud EBD. These sediments, however, are readily identifiable and are not easily confused with sediments of adjacent depositional zones.

Applicability of Airy Deepwater Wave Theory

Airy deepwater wave theory provides estimates of maximum horizontal velocity at the bottom with an error of less than 5% for sites where depth (h) > 0.25 deepwater wavelength (L) (Komar 1976):

$$(1) \quad h > L/4.$$

In order to evaluate the applicability of deepwater wave theory to our data, estimates of deepwater wavelength are necessary because measurements are unavailable for most lakes. Deepwater wavelength is approximately 20 times wave height (H) in lakes (Wetzel 1983; U.S. Army Coastal Engineering Research Center 1984):

$$(2) \quad L = 20H.$$

Measurements of wave height are also unavailable for most lakes, but maximum possible wave height is related to maximum fetch (Wetzel 1983; U.S. Army Coastal Engineering Research Center 1984). Maximum deepwater wave height ($H_{\max}$, metres) can be estimated by the empirical relationship

$$(3) \quad H_{\max} = 0.332F^{0.5}$$

where F = maximum fetch (kilometres) (Wetzel 1983). Substituting equation 3 for wave height in equation 2, we obtain deepwater wavelength as a function of maximum fetch:

$$(4) \quad L = 6.640F^{0.5}.$$

Substituting equation 4 for wavelength in equation 1 and simplifying, one obtains the limit of applicability of Airy deepwater theory (less than 5% error) in terms of site depth (metres) and maximum fetch (kilometres):

$$(5) \quad h > 1.660F^{0.5}.$$

Evaluating equation 5 for our data set shows that under maximum wave conditions, Airy deepwater wave theory gives acceptable results at 88% of the sites (98% of the fine-grained

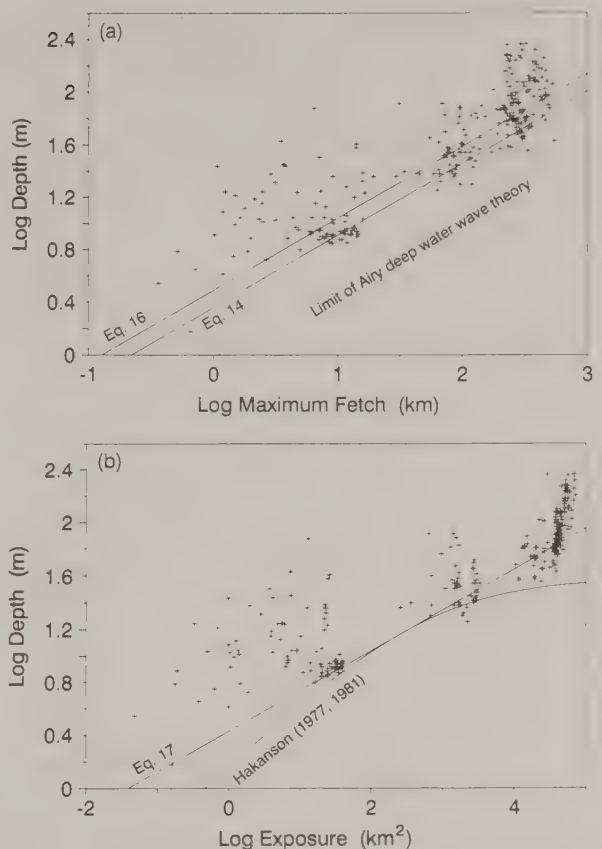


FIG. 3. (a). Plot of depth versus maximum fetch, with observed mud DBDs (+) and theoretical (equation 16, $\log \text{ mud EBD} = 0.488 + 0.549 \log F$) and empirical (equation 14, $\log \text{ mud EBD} = 0.360 + 0.549 \log F$) equations defining the mud EBD. Also plotted is the line defining the limit of applicability of Airy deepwater wave theory. (b). Plot of depth versus exposure, with observed mud DBDs (+) and empirical estimates of the mud EBD from this study (equation 17, $\log \text{ mud EBD} = 0.429 + 0.305 \log E$) and Håkanson (1977, 1981). Most mud DBDs lie near or above the empirical mud EBDs which define an upper limit to the distribution of mud in lakes.

sites, 72% of the coarse-grained sites) and all of the mud DBDs (Fig. 3a). There are no shallow-water sites in our data set ($h < L/20$), and most of the intermediate water sites are coarse-grained (91%). Errors associated with overestimates of maximum horizontal velocities at these coarse-grained intermediate water sites should have no effect on our results because virtually all of the fine-grained sites and mud DBDs are at least 1.5-fold deeper than these sites and the limits of Airy deepwater wave theory (Fig. 3a).

Simplifying Airy Deepwater Wave Theory

The maximum horizontal velocity (metres per second) at the bottom is described by

$$(6) \quad U_m = \frac{\pi H}{T \sinh(2\pi h/L)}$$

where H = wave height (metres), h = water depth (metres), L = wavelength (metres), and T = wave period (seconds) (Airy 1845; Komar 1976). We have already expressed maximum wave height and deepwater wavelength as functions of maxi-

imum fetch (equations 3 and 4). Wave period can also be expressed in terms of maximum fetch because wave period and wavelength are related theoretically in deep water:

$$(7) \quad T = (2\pi L/g)^{0.5}.$$

Substituting equation 4 for wavelength in equation 7 and simplifying yields deepwater wave period as a function of maximum fetch:

$$(8) \quad T = 2.061F^{0.25}.$$

Substituting equations 3, 4, and 8 for wave height, wavelength, and wave period in equation 6 and simplifying yields U_m at the bottom, generated by maximum wave conditions in deep water, as a function of maximum fetch and water depth:

$$(9) \quad U_m = \frac{0.506F^{0.25}}{\sinh(0.946h/F^{0.5})}.$$

With this relationship, one can estimate U_m for deepwater sites in lakes.

Simplifying Particle Threshold Theory

Threshold velocity (U_t) for particles under oscillatory waves can be expressed as

$$(10) \quad \frac{\rho U_t^2}{(\rho_s - \rho)gD} = 0.21(d_o/D)^{0.5}$$

where ρ = fluid density, ρ_s = particle density, $g = 9.8 \text{ m}\cdot\text{s}^{-2}$, D = particle diameter (metres) and d_o = orbital diameter (metres) (Komar and Miller 1975). Assuming $\rho = 1000 \text{ kg}\cdot\text{m}^{-3}$, $\rho_s = 2650 \text{ kg}\cdot\text{m}^{-3}$, setting $D = 2.3 \times 10^{-5} \text{ m}$, and solving for U_t (metres per second), one obtains threshold velocity for 23 μm particles as a function of orbital diameter:

$$(11) \quad U_{t23} = 0.128d_o^{0.25}.$$

Near-bottom orbital diameter in deep water can be expressed as (Airy 1845; Komar and Miller 1975)

$$(12) \quad d_o = \frac{H}{\sinh(2\pi h/L)}.$$

Substituting equation 3 for wave height and equation 4 for wave length in equation 12 and then substituting for d_o in equation 11 and simplifying, one obtains U_{t23} under maximum wave conditions in deep water as a function of maximum fetch and depth:

$$(13) \quad U_{t23} = \frac{0.097F^{0.125}}{(\sinh(0.946h/F^{0.5}))^{0.25}}.$$

Both U_m and U_{t23} for maximum wave height in deep water can now be estimated from only site depth and maximum fetch.

Mud EBD: Upper Limit to the Distribution of Mud in Lakes

It is now possible to estimate U_m (equation 9) and U_{t23} (equation 13) for each site and compare the wave energy and texture classifications. At 78% (608/783) of the fine-grained sites, U_m is less than U_{t23} , and the energy and texture classifications are in agreement. At 68% (320/477) of the coarse-grained sites, U_m is greater than U_{t23} , and the energy and texture classifications are also in agreement. However, in our data set, fine-grained sediments should not occur in environments where $U_m > U_{t23}$ and wave energy is capable of transporting and depositing sand. Sites in such environments should have coarse-

grained sediments that reflect the vigorous interaction of waves with the bottom. Thus, our assumption of maximum wave height appears to have overestimated energy conditions responsible for sediment distribution.

An empirical mud EBD was determined with nonlinear iterative regression using the variables site depth and maximum fetch:

$$(14) \quad \text{Empirical mud EBD} = 2.291F^{0.549}.$$

A comparison of site depth with the empirical mud EBD (equation 14) shows that 89% (700/783) of the fine-grained sites occur deeper than the empirical mud EBD, while only 55% (261/477) of the coarse-grained sites occur shallower than the empirical mud EBD. Although defining a better upper limit to the distribution of mud than the theoretical fit based on maximum wave height, the empirical mud EBD leaves many coarse-grained sites at depths greater than the mud EBD.

By equating equations 9 and 13 and solving for depth, one obtains the theoretical mud EBD for maximum wave conditions as a function of maximum fetch, permitting a direct comparison of the theoretical and empirical relationships:

$$(15) \quad \text{Theoretical mud EBD} = 1.057F^{0.5} \sinh^{-1}(9.052F^{0.167}).$$

Equation 15 can be further simplified through a log-log regression ($r^2 = 1.00$) to a form comparable with the empirical equation:

$$(16) \quad \text{Theoretical mud EBD} = 3.076F^{0.549}.$$

A plot of the mud DBDs against maximum fetch and the logarithmically transformed theoretical (equation 16) and empirical (equation 14) mud EBDs is shown in Fig. 3a. The empirical mud EBD (equation 14) is parallel to the theoretical mud EBD (equation 16), but the intercept for the empirical relationship is shallower than the theoretical intercept. The empirical relationship provides an upper boundary to the distribution of mud DBDs but fails to account for the frequent occurrence of many mud DBDs at depth.

Similar results were obtained with exposure (square kilometres) as a surrogate for wave height. The empirical relationship between exposure and the mud EBD was determined by nonlinear iterative regression:

$$(17) \quad \text{Empirical mud EBD} = 2.685E^{0.305}.$$

This relationship correctly classifies 90% (701/783) of the fine-grained sites and 59% (283/477) of the coarse-grained sites. A graphical presentation of mud DBDs against exposure and empirical mud EBDs (equation 17) is shown in Fig. 3b. As with the empirical fetch relationship (Fig. 3a), few mud DBDs occur shallower than the mud EBD, but many occur much deeper (Fig. 3b). The only other empirical approach attempted, that of Håkanson (1977, 1981), is also plotted in Fig. 3b. The mud EBD, as we have defined it, occurs approximately in the middle of the Håkanson's (1977, 1981) zone of transportation. Therefore, the line we have plotted is the mean of the two equations that define Håkanson's (1977, 1981) zone of transportation. Effective fetch has been replaced by the square root of exposure because these terms are nearly identical mathematically. The predictions of Håkanson (1977, 1981) are accurate for Lake Vänern, the lake from which the model was generated, but do not adequately describe sediment distribution in the other lakes (Fig. 3b). While this curve correctly classifies 96% (749/783) of the fine-grained sites, it correctly classifies only 34% (163/477) of the coarse-grained sites, which again often occur at depths greater than the mud EBD.

Estimating Critical Wave Heights from Sediment Distribution

While the theoretical mud EBD was estimated from the maximum wave height, the shallower depth of the empirical mud EBD suggests that some submaximal (more frequent) wave determines the position of this boundary. The submaximal or critical wave height (H_{crit}) of the empirical result can be estimated using nonlinear regression (Wilkinson 1989). H_{crit} can then be compared with H_{max} and wave height exceedances available for Lakes Superior and Huron. Not only will this comparison improve estimates of U_m and U_{t23} , it will also provide important first estimates of the frequency of severe events responsible for sediment distribution.

Critical wave height (H_{crit}) as a function of maximum fetch was estimated by nonlinear regression as

$$(18) \quad H_{crit} = 0.254F^{0.5}.$$

Replacing equation 3 with equation 18, we derive equations for U_m and U_{t23} that reflect critical wave conditions, rather than maximum wave conditions:

$$(19) \quad \text{Critical } U_m = \frac{0.442F^{0.25}}{\sinh(1.237h/F^{0.5})}$$

$$(20) \quad \text{Critical } U_{t23} = \frac{0.091F^{0.125}}{(\sinh(1.237h/F^{0.5}))^{0.25}}.$$

Critical wave height was also estimated as a function of exposure by nonlinear regression:

$$(21) \quad H_{crit} = 0.293E^{0.278}.$$

Replacing equation 3 with equation 21, allows equations to be derived for U_m and U_{t23} as a function of exposure and depth that reflect critical wave conditions:

$$(22) \quad \text{Critical } U_m = \frac{0.475E^{0.139}}{\sinh(1.072h/E^{0.278})}$$

$$(23) \quad \text{Critical } U_{t23} = \frac{0.094E^{0.0695}}{(\sinh(1.072h/E^{0.278}))^{0.25}}.$$

For any depth on the empirical mud EBDs (equations 14 and 17), equations 18 and 21 predict identical critical wave heights. Therefore, U_m and U_{t23} at the mud EBD predicted using either maximum fetch (equations 19 and 20) or exposure (equations 22 and 23) are identical. This remarkable agreement between empirical fits to the observed sediment distribution using two different energy surrogates provides compelling support for the empirical analysis. Mud EBDs of 1, 3, 9, 27, and 81 m correspond to maximum fetches of 0.22, 1.6, 12.1, 89.5, and 661 km and exposures of 0.04, 1.4, 52.8, 1935, and 70 950 km², respectively. The threshold velocities for 23 μ m particles at the mud EBD for critical wave height range from 0.043 to 0.092 m·s⁻¹ and are in excellent agreement with the estimates of Komar and Miller (1975).

Critical wave height is approximately 77% of maximum wave height (estimated from maximum fetch, equations 3 and 18). Since the maximum wave height for a given fetch or exposure is very infrequent (10 to 15 yr) (Saulesleja 1986), and well beyond the time needed for cohesion of fine-grained sediments, it is not surprising that a submaximal wave provides a superior estimate of the upper limit to the distribution of mud. As wave height exceedance tables are available for Lakes Superior and Huron (Saulesleja 1986), it is possible to evaluate the frequency of occurrence of critical wave heights (equations 18 and 21). Critical wave height is exceeded approximately 0.3% of the

time in Lake Superior, or approximately 1 d·yr⁻¹. In Lake Huron, critical wave height is exceeded 0.4% of the time, or approximately 1.5 d·yr⁻¹. In both lakes, exceedance of critical wave height is more likely in late fall and winter isothermal periods than at other times of the year (Saulesleja 1986). This suggests that the several biggest storm events each year are responsible for sediment redistribution and that extremely rare events need not be invoked to explain sediment distribution in lakes.

Effects of Slope

Since over 40% of the coarse-grained sediments in our data occur deeper than the mud EBD, usually on slopes greater than 3%, we hypothesize that slope can effectively lower particle threshold velocity either by reducing deposit or grain stability or increasing wave interaction with the bottom by compressing wave orbital diameter. There are few theoretical relationships for U_m or U_t that include slope, and those that exist are very complex and data demanding, requiring detailed site sediment descriptions (Komar 1976; Wiberg and Smith 1987). Therefore, to test our hypothesis, we used nonlinear iterative regression to estimate the apparent threshold velocity (U_{ta23}) from U_m and slope for 23 μ m particles at all sites deeper than the mud EBD.

At sites deeper than the mud EBD, slope was found to lower threshold velocity exponentially:

$$(24) \quad U_{ta23} = 10^{(6 - 2.40\text{slope})}.$$

For sites deeper than the mud EBD, comparisons of U_{ta23} (equation 24) and U_m , estimated from maximum fetch for the critical wave height in deep water (equation 19), correctly classify 92% (643/700) of fine-grained sites ($U_m < U_{ta23}$) and 51% (110/216) of coarse-grained sites ($U_m > U_{ta23}$). For all sites, the predictions of theory using maximum fetch (equations 19, 20 and 24) correctly classify 82% (643/783) of fine-grained sites and 78% (371/477) of coarse-grained sites.

Comparisons of U_m estimated from exposure (equation 22) and U_{ta23} (equation 24) show that U_m is less than U_{ta23} at 91% (641/701) of the fine-grained sites below the mud EBD and that U_m exceeds U_{ta23} at 59% (115/194) of the coarse-grained sites below the mud EBD. Overall, predictions of theory for exposure and critical wave height (equations 22, 23, and 24) correctly classify 82% of fine-grained sites (641/783) and 83% (398/477) of coarse-grained sites.

The relationship between U_{ta23} (equation 24) and U_m (equations 19 and 22) can be illustrated by plotting a family of curves for slope with mud DBD versus either maximum fetch or exposure (Fig. 4). We have limited the family of curves to the range of our data set, which covers most situations encountered in lakes. For slopes of 3% or less, the mud EBD is equivalent to the mud DBD. Slopes of greater than 3% have significant modifying effects on sediment distribution. Slope effects are greatest for slopes between 3 and 5% and decrease in magnitude as slope increases. Figure 4 shows that in large lakes (maximum fetch > 50 km or exposure > 1000 km²), fine-grained sediments are rarely found on slopes greater than 7.5%, and in small lakes (maximum fetch < 1 km or exposure < 1 km²), fine-grained sediments are seldom found on slopes greater than 15%.

These results can also be summarized by comparing observed mud DBDs with the theoretical-empirical predictions (Fig. 5). In Fig. 5, many observed mud DBDs equal predicted mud DBDs, and most predicted mud DBDs lie close to the expected 1:1 relationship. Comparing Fig. 5 with Fig. 3, it is obvious

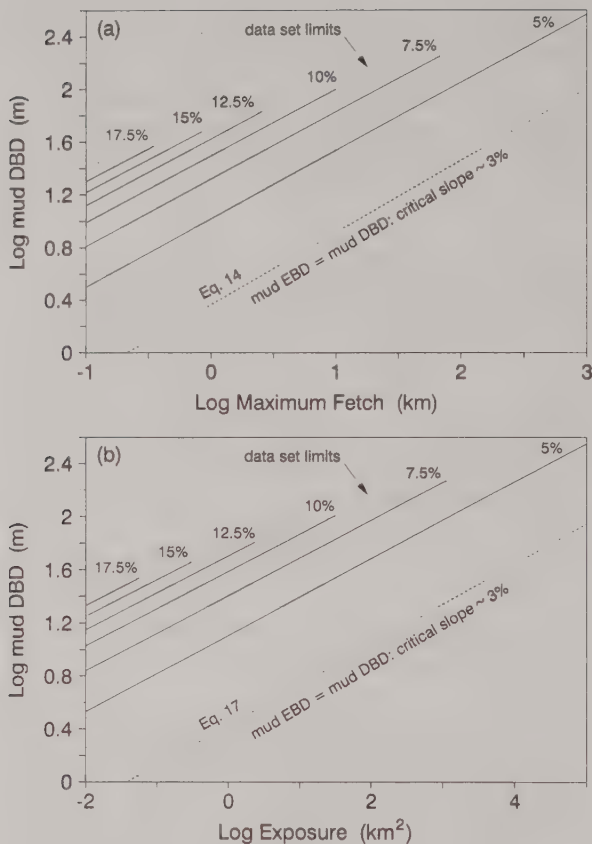


FIG. 4. Family of curves that predict the mud DBD as a function of slope and either (a) maximum fetch or (b) exposure.

that slope effects explain why most of the mud DBDs (and much coarse-grained sediments) are located at water depths much greater than the mud EBD estimated only from wave and particle threshold theory. Log-linear regressions of observed versus predicted mud DBDs show that the predictions explain 89% (maximum fetch, $\log \text{ mud DBD} = -0.081 + 1.076 \log \text{ mud DBD}_{\text{pred}}$, $r^2 = 89\%$, $\text{SE}_{\text{est}} = 0.151$, $n = 254$) and 93% (exposure, $\log \text{ mud DBD} = -0.045 + 1.028 \log \text{ mud DBD}_{\text{pred}}$, $r^2 = 93\%$, $\text{SE}_{\text{est}} = 0.121$, $n = 254$) of the variation in mud DBDs for our data set. Exposure turns out to be a better predictor of mud DBDs than maximum fetch (Fig. 5) because exposure is less variable in lakes than maximum fetch (note the more even distribution of maximum fetch in Fig. 3a as opposed to the bunched distribution of exposure in Fig. 3b). In our data, there is no evidence that midlake sites, with lower maximum fetch than sites at either end of the lake (but with similar exposures), have mud DBDs (or mud EBDs) that are shallower than those at either end of the lake.

A completely empirical approach, estimating the mud DBD (metres) from either maximum fetch (kilometres) or exposure (square kilometres) and slope (percent) using nonlinear logistic regression (Wilkinson 1989), results in predictions similar to those obtained from theory:

$$(25) \quad \log \text{ DBD} = -0.107 + 0.742 \log F + 0.0653 \text{ slope}$$

$$(26) \quad \log \text{ DBD} = 0.123 + 0.370 \log E + 0.0526 \text{ slope.}$$

For fine-grained sites, 87% (683/783) are correctly classified by equation 25, as are 70% (334/477) of coarse-grained sites.

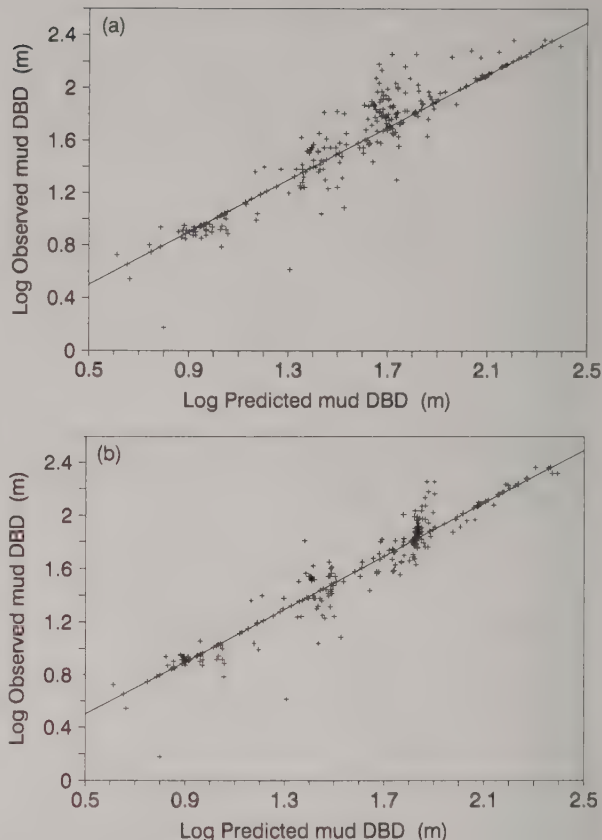


FIG. 5. Observed versus predicted mud DBDs using (a) maximum fetch or (b) exposure as the wave energy surrogate. Lines represent the 1:1 relationship.

When exposure is used as an energy surrogate, 87% (683/783) of fine-grained sites are correctly classified by equation 26 as are 76% (362/477) of coarse-grained sites. In both theoretical-empirical (equation 24) and empirical (equations 25 and 26) relationships the effect of slope decreases as depth increases and exposure or maximum fetch decreases.

Conclusion

With a large data set representative of lakes of all sizes and depths, we have provided a general test of wave and sediment threshold theory as predictors of sediment distribution. Observed sediment distribution indicates that the critical wave heights responsible for redistribution of sediments in lakes are approximately 77% of maximum wave heights and that these wave conditions occur during the one or two biggest storms each year. Either exposure or maximum fetch may be used as a wave energy surrogate, although relationships with exposure yield slightly, but consistently better predictions. The reduction of theoretical relationships to simple equations requiring only site depth and either exposure or maximum fetch demonstrates that wave energy provides an upper limit to the distribution of fine-grained sediment in lakes (mud EBD) but that most mud DBDs and over 40% of coarse-grained sediments occur considerably deeper than this boundary, especially if the slope is greater than 3%.

Slopes of less than 3% have little or no effect on sediment distribution, while fine-grained sediments are rarely found on

slopes greater than 10%. Slope either reduces particle threshold velocity (or reduces sediment stability) or increases wave interaction with the bottom. Theoretical-empirical and empirical models that include slope explain most of the variation in mud DBDs deeper than the mud EBD. These results agree with estimates of slope effects from a single basin (Håkanson 1977, 1981) and the distribution of fine-grained sediment on continental slopes, where slope rarely exceeds 10% (Bouma 1983).

The theoretical-empirical and empirical models that include slope can be used to estimate the area of the deposition zone from lake morphometry. Such estimates have important applications for the calculation of whole-lake sedimentation rates, resuspension rates, and areas of sediment focusing. The accurate prediction of the deposition zone using the mud DBD theory should greatly simplify mass-balance modelling of particulate matter and associated contaminants.

The effects of slope on sediment distribution have biological implications. For example, Duarte and Kalff (1986) found significantly less macrophyte biomass on slopes greater than 2.2 to 5.3% and hypothesized that this may be due to substrate type. Slope has also been shown to be an important predictor of zoobenthic biomass in lakes (Rasmussen and Kalff 1987; Rasmussen 1988a, 1988b). It is also possible that slope influences other biota dependent on substrate quality, such as spawning fish.

Our results also suggest that the theory of waves and sediment thresholds is only appropriate or accurate for many sites in lakes when slope is considered. The effects of slope have received little attention due to the emphasis of sedimentological research on continental shelves where slopes are negligible and coarse-grained sediments predominate. In lakes, sedimentary environments are dominated by fine-grained sediments, and sloped sites are often the only sedimentary environments with coarse-grained sediments.

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Nutrient Regeneration by Sockeye Salmon (*Oncorhynchus nerka*) Fry and Subsequent Effects on Zooplankton and Phytoplankton

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Schindler, D. E. 1992. Nutrient regeneration by sockeye salmon (*Oncorhynchus nerka*) fry and subsequent effects on zooplankton and phytoplankton. *Can. J. Fish. Aquat. Sci.* 49: 2498–2506.

Planktivorous fish can affect phytoplankton indirectly by control of herbivore communities, and directly by excretion of soluble nutrients. A field mesocosm study showed that nutrient regeneration by sockeye salmon (*Oncorhynchus nerka*) fry substantially increased biomass of zooplankton and phytoplankton relative to fishless controls. Plankton communities in enclosures treated with only fish-regenerated nutrients but not fish predation responded differently to two densities of fish. Enclosures with low fish densities exhibited significant increases in zooplankton biomass and small increases in phytoplankton biomass. Nutrients resulting from high fish densities stimulated blooms of ungrazable *Dinobryon*, and extremely low zooplankton biomass when compared with fishless controls. Phosphorus excretion rates by zooplankton and fish explained 55% of the variance in total plankton biomass in predation-free enclosures and 95% of the variance in chlorophyll *a* concentrations for enclosures with predators and fish-regenerated nutrients. The results of this experiment suggest that algal production is greatly enhanced by excretion of nutrients by fish, while grazer control is less important.

Les poissons planctivores peuvent influencer indirectement sur le phytoplancton en contrôlant les communautés herbivores, et directement en excréant des éléments nutritifs solubles. Une étude des mésocosmes sur le terrain a montré que la régénération des éléments nutritifs par les alevins de saumon rouge (*Oncorhynchus nerka*) a accru substantiellement la biomasse du zooplancton et du phytoplancton par rapport à des milieux témoins sans poissons. Des communautés planctoniques en espace clos traitées uniquement avec des éléments nutritifs régénérés par les poissons, mais sans prédation, ont réagi différemment à deux densités de poissons. Les espaces clos à faible densité de poissons ont montré des augmentations significatives de la biomasse de zooplancton et de faibles augmentations de la biomasse de phytoplancton. Les éléments nutritifs produits par des densités élevées de poissons ont stimulé le développement de *Dinobryon* non broutable et ont produit une biomasse extrêmement faible de zooplancton par rapport à des milieux témoins sans poissons. Les taux d'excrétion du P du zooplancton et du poisson expliquent 55 % de la variance de la biomasse totale de plancton dans des espaces clos sans prédation et 95 % de la variance des concentrations de chlorophylle *a* dans les espaces clos avec prédateurs et éléments nutritifs régénérés par les poissons. Les résultats de cette expérience indiquent que la production alguaire est grandement accrue par l'excrétion d'éléments nutritifs des poissons, alors que le contrôle des brouteurs est moins important.

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Intense predation by fish on large-bodied zooplankton favours zooplankton communities dominated by small-bodied species and individuals (Hrbacek et al. 1961; Brooks and Dodson 1965). In some cases, fish reduce the overall zooplankton biomass in lakes (Evans 1986). The effects that fish have on zooplankton also affect phytoplankton biomass and productivity (Hrbacek et al. 1961; Shapiro 1980; Carpenter et al. 1987). Reductions in both zooplankton average size (Peters and Downing 1984) and overall community biomass (Henrikson et al. 1980; Vanni 1987) will result in reduced grazing pressure exerted on the phytoplankton community, allowing it to increase in biomass and productivity (Carpenter et al. 1987). This indirect mechanism has been used to explain why dense populations of zooplanktivorous fish often cooccur with relatively high phytoplankton biomass (Carpenter et al. 1987; McQueen et al. 1989). Similar studies in rivers have shown parallel evidence of strong fish effects on lower trophic levels (Power 1990).

Recent evidence suggests that an enhanced phytoplankton response may be due to increased nutrient availability from fish (Braband et al. 1990; Reinertsen et al. 1990; Vanni and Findlay 1990). These studies are in disagreement with older evidence which suggests that fish do not contribute substantially to internal nutrient cycling in lakes (Kitchell et al. 1975; Nakashima and Leggett 1980).

Fish may increase internal nutrient loading to phytoplankton in several ways. Fish excreta and feces may be direct sources of usable nutrients (Lamarra 1975; Kraft 1991). Fish can also increase nutrient excretion by the zooplankton community because small zooplankton have higher mass-specific excretion rates than larger forms (Peters and Rigler 1973). A small-bodied zooplankton community resulting from fish predation will therefore excrete greater quantities of soluble nutrients than a large-bodied community of equal biomass (Bartell and Kitchell 1978). Fish mortality and the resulting decay of fish carcasses are also a potential source of nutrients to phytoplankton (Threlkeld 1987, 1988).

Because the predation and nutrient enhancement described above affect natural phytoplankton communities commensur-

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ately, little empirical evidence exists for comparison of the relative impact of each on phytoplankton. In this paper, I describe the results of a replicated field enclosure experiment designed to assess the relative impact of increased nutrient regeneration caused by fish on phytoplankton, in isolation of indirect predation effects. My enclosures were divided into compartments separated by screens that allowed nutrient exchange between compartments. I reasoned that if planktivorous fish were added to one of these compartments, nutrient released into the water could increase phytoplankton biomass in the other compartment of the enclosure. Because the zooplankton in the fishless compartments of these enclosures would not be controlled by fish predation, they would be the eventual beneficiaries of this increase in phytoplankton production.

Materials and Methods

Study Site

This experiment was conducted from July to late August 1989 in a shallow pond approximate mean depth 1 m) connecting Chilko Lake (51°N, 124°W, elevation 1172 m) (Stockner and Shortreed 1983) with a small lake (Little Lagoon, approximate surface area 10 ha) on the eastern edge of the coastal mountains of British Columbia.

Little Lagoon and its connecting pond have substantial resident populations of reidside shiners (*Richardsonius balteatus*) and mountain whitefish (*Prosopium williamsoni*). Large numbers of fry of sockeye salmon (*Oncorhynchus nerka*) invade the Little Lagoon and pond from Chilko Lake early in June of each summer. These fish were abundant in the Little Lagoon and connecting pond throughout the course of this study.

The crustacean zooplankton community of the Little Lagoon was composed of the cladocerans *Ceriodaphnia reticulata*, *Daphnia rosea*, *Bosmina longirostris*, and several *Chydorus* spp. and the cyclopoid copepods (*Diacyclops thomasi* and *Acanthocyclops vernalis*). The rotifer community is primarily composed of *Keratella* spp. and *Polyarthra* spp., but *Trichocerca* spp., *Lepadella* spp., *Kellicottia* spp., and *Asplanchna* spp. were also all present at low densities.

Enclosure Construction

In order to study the effects of nutrient regeneration by planktivorous fish on zooplankton and phytoplankton communities, it was necessary to isolate the nutrient recycling effects of planktivory by fish from direct predation effects. Unlike previous mesocosm studies assessing the effects of fish planktivory on plankton communities (McQueen et al. 1986; O'Neill and Hyatt 1987; Vanni 1987; Vanni and Findlay 1990), I divided my enclosures into two compartments of equal size using screens. These allowed passive flow of nutrients between the compartments of each enclosure. Nutrients regenerated from fish placed on one side of the barrier could potentially affect plankton on the opposite side without directly exposing those plankton to fish predation.

Each enclosure was constructed of transparent, 6-mil polyethylene sheeting stretched around eight wooden posts, driven into the sediment in a square arrangement (3 × 3 m) and 1 m of water. The bottom edge of each enclosure wall was buried approximately 20 cm into the sediments, and the top edge was pulled approximately 50 cm above the water level. The top of each wall could be lowered below the water level to flush the enclosures with lake water immediately preceding the initiation

of the experiment. Each of these enclosures contained 9 m³ of water, exposed to the sediments.

Two panels (49 × 123 cm) of 116-μm-mesh Nitex were sewn into a wall of 6-mil polyethylene sheeting that was then used to divide each of the enclosures into two compartments of equal volume. This mesh size was a compromise between the need to facilitate nutrient exchange between the two compartments of each enclosure and to impede the exchange of larger phytoplankton, zooplankton, and fish. Finer mesh would have been quickly clogged with periphyton and coarser mesh would have allowed juvenile zooplankton to pass easily. I assumed that these screens sufficiently impeded movement of rotifers and phytoplankton to successfully separate the plankton communities on either side of the dividing wall.

Experimental Design

Three treatment levels were randomly assigned to eight divided enclosures. Two sockeye fry were added to one compartment in each of three enclosures ("low-fish"), four sockeye fry to an additional two enclosures ("high-fish"), and two enclosures were left fishless ("no-fish"). I will refer to the two compartments of each enclosure as either the "fish" or "fishless" compartment.

The sockeye fry averaged approximately 4 g. The high-fish treatment corresponded to approximately 3.56 g fish·m⁻³ and the low-fish treatment to approximately 1.78 g fish·m⁻³. Fish were caught by beach seine in the Little Lagoon and added to the enclosures on July 22, 1989, after flushing the enclosures with lake water. The health of fish was visually monitored following their addition. Only one death was recorded and this fish was removed and replaced 1 d after it was put in the compartment. All other fish lived for the duration of the study. No invasions into the enclosures by fish from the Little Lagoon were observed.

Sampling Methods

All enclosures and the Little Lagoon were sampled 2 d before and 7, 24, and 32 d after the addition of the sockeye fry. Zooplankton were sampled by pumping 120–150 L of water from a depth of 50 cm (at the center of each enclosure compartment) through a 2.5-cm inside diameter plastic hose with a 12-V DC diaphragm bilge pump. The total volume of water pumped was determined with an in-line, unidirectional flow meter (100% pump sampling efficiency was assumed). The samples were concentrated with a 20-μm-mesh plankton net and then preserved in a 4% solution of formalin–sucrose buffered with Borax (Haney and Hall 1973). Water for all other plankton and chemical analyses was collected as an integrated sample in opaque, acid-washed polyethylene bottles from the same location in the enclosures. Phytoplankton were preserved in a 100-mL subsample of this water using Lugol's iodine solution. Phototrophic picoplankton samples were preserved by filtering 5 mL of the enclosure water through a 0.2-μm Nuclepore filter stained with Irgalan black (MacIsaac and Stockner 1985). These filters were then air dried and stored in opaque Petri dishes. Chlorophyll *a* concentrations were determined by filtering 500 mL of sample water through 0.8-μm pore size Millipore AA filters. Filters were then macerated in 90% acetone and the amount of chlorophyll fluorescence (corrected for phaeopigments) determined with a Turner model III fluorometer (Stephens and Brandstaetter 1983). Periphytic chlorophyll *a* on the inside of the enclosure walls was sampled by cutting three discs (3 cm in diameter) of polyethylene from the enclosure

walls after the outside face had been scrubbed free of algae. These discs were then treated in the same fashion as the Millipore AA filters to determine the concentration of chlorophyll on the inside of the enclosure walls.

Zooplankton samples were enumerated for crustaceans with a Wild stereoscope at 25× magnification. Subsamples were progressively enumerated until at least one third of the entire sample was observed or until at least 150 individuals of the most abundant taxa had been counted. Size distributions were obtained for the four most abundant taxa in each sample using an ocular micrometer to measure the length of the first 30 individuals encountered from each taxon. Cladoceran total length was determined as the distance between the anterior tip of the head to the insertion of the caudal spine (McCauley 1984). The length of copepods was determined as the distance between the anterior tip of the head to the posterior tip of the caudal rami excluding the furcal rami (McCauley 1984). Dry-mass estimates of the four most abundant zooplankton taxa were determined using published length–mass regression equations (McCauley 1984). Mass of *D. rosea* was estimated using an equation for closely related *D. longiremis*.

Rotifers were enumerated by subsampling each sample with a wide-bore repeating pipette and then enumerating in a Sedgewick-Rafter cell using a Wild compound microscope at 50×. Subsamples were progressively enumerated until a total of at least 250 individuals was encountered. Rotifer biomass was calculated using the equations of McCauley (1984) and the average dimensions of the rotifers in each sample (obtained with an ocular micrometer). I assumed that dry mass equalled 0.05 times the biovolume (Schindler and Noven 1971).

Phytoplankton were enumerated with a Nikon inverted microscope fitted with phase-contrast optics. Fifty-millilitre samples were settled in counting chambers and then 40 haphazardly chosen fields were enumerated. Phytoplankton biomass estimates were determined by assuming that dry mass is equal to 0.3 times their biovolume (Walters et al. 1987). Biovolumes were estimated by converting linear dimensions (obtained with an ocular micrometer) to the volume of equivalently shaped geometric bodies.

Phototrophic picoplankton (cells <2 µm diameter) were identified and enumerated as described by MacIsaac and Stockner (1985) with a Zeiss epifluorescent microscope at 1250× magnification. A minimum of 30 haphazardly chosen fields were enumerated from each sample.

Estimation of Excretion Rates

Fish P excretion rates were estimated with a fish bioenergetics model (Hewett and Johnson 1987; Kraft 1991). Sockeye salmon physiological parameters reported by Beauchamp et al. (1989) were used in these calculations. Growth rates of the sockeye fry in the enclosures could not be calculated because I was unable to recapture them at the end of the experiment. P excretion rates were calculated based on the assumption that growth rates were 0%. Other parameters used were an average ambient temperature of 18°C, a P assimilation efficiency of 72%, and a total P content of 0.005 g P·g wet mass fish⁻¹.

I estimated zooplankton P excretion rates using the models of Peters and Downing (1984) in the same manner as Vanni and Findlay (1990). Calculation of food consumption rates for zooplankton can provide estimates of excretion rates because approximately 50% of ingested P is excreted (Peters 1975). Rotifer ingestion rates were calculated using the equation

$$\log I = -1.353 + 0.544 \log W + 0.577 \log S - 0.0002M + 0.466 \log R + 0.00006C - 0.045 (\log R)^2$$

where I = individual ingestion rate (micrograms per wet mass of animal per day), W = animal mass (micrograms per individual), S = food concentration (parts per million wet mass), and R = mean food particle volume (cubic micrometres), W , S , and R were obtained using data from individual enclosure compartments. Because over 90% of the grazable (i.e. all algae except colonial *Dinobryon*) phytoplankton biomass was represented by assuming that the zooplankton were grazing entirely on *Chlamydomonas*, these calculations were simplified by assuming that the zooplankton were grazing entirely on *Chlamydomonas*. The values for C (volume of the experimental vessel) and M (duration of experiment) were represented by the mean values given by Peters and Downing (1984). These last two parameters are used in short-term laboratory experiments but are not important in long-term enclosure experiments (Vanni and Findlay 1990).

Cladoceran ingestion rates were calculated similarly using the equation

$$\log I = -1.343 + 0.486 \log S + 0.587 \log W + 0.014Ca - 0.165 (\log R)^2 + 0.887 \log R + 0.027T$$

where T = temperature (18°C) and Ca = 11.0 (Peters and Downing 1984). Individual ingestion rates were multiplied by population densities for each zooplankton taxon to obtain population ingestion rates that were summed to obtain community ingestion rates for each compartment. Carbon ingestion rates were estimated by assuming that C accounts for 10% of the wet mass of ingested material (Peters and Downing 1984). P ingestion was estimated as 1% of the C ingestion and 50% of this was assumed to be excreted (Vanni and Findlay 1990).

Statistical Methods

Statistical analyses were aided with the use of the Systat computer package (Wilkinson 1989). Nonparametric analyses were performed to determine whether significant differences existed between the treatment groups before the initiation of the experiment, and over the course of the experiment. In order to do this, each compartment of the two no-fish enclosures were randomly designated as a “fish” compartment or a “fishless” compartment.

The Kruskal–Wallis ANOVA was used to determine whether the enclosures were equivalent at the start of the experiment. Densities of all dominant (>5% of individuals) zooplankton and phytoplankton groups were compared using this method.

A Friedman’s two-way ANOVA for related samples was used as a nonparametric alternative to the repeated measures ANOVA (Sokal and Rohlf 1981) to determine whether significant treatment effects existed over the course of the experiment. As this analysis does not allow the use of unbalanced experimental designs, I randomly omitted one replicate from the low-fish treatment. Data for each variable to be tested were placed in blocks relating them by sampling date. The data in every block were then ranked across treatments and the sums of the rankings for each treatment (over the course of the experiment) were compared with the Friedman’s test statistic (χ^2) based on replicated samples (Zar 1984). A nonparametric alternative to the Tukey test that used data from the Friedman’s test calculation was used as a multiple comparison test.

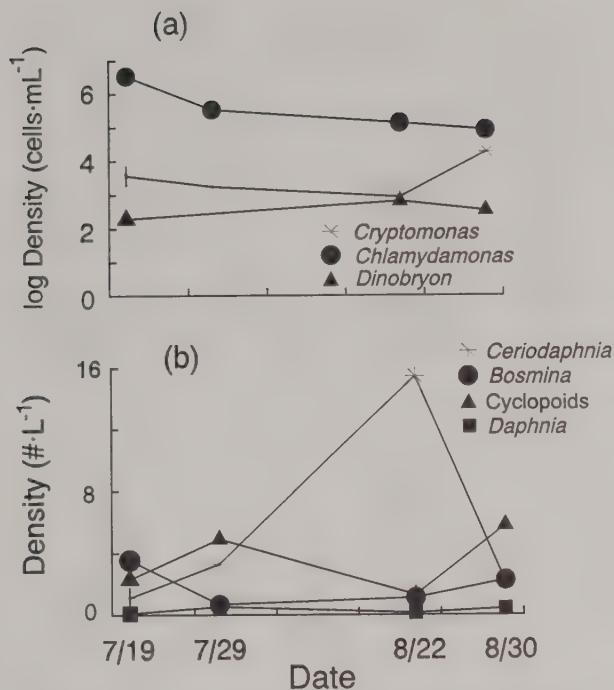


FIG. 1. Dynamics of dominant (a) phytoplankton and (b) zooplankton in the Little Lagoon over the course of the study. Means of two replicate samples are represented.

Results

Little Lagoon dynamics

Chlamydomonas spp. and *Cryptomonas* spp. dominated (80–85% by numbers) the phytoplankton community in the Little Lagoon throughout the study (Fig. 1a). Many other taxa were present at all sampling times including a colonial *Dinobryon* spp., *Scenedesmus* spp., *Arthrodesmus* spp., and a variety of small desmids, diatoms, and ciliates. Each of these groups except *Dinobryon* remained at very low densities (<2% of total). *Dinobryon* densities remained relatively unchanged over the course of the experiment in the Little Lagoon (Fig. 1a).

Phototrophic picoplankton (Stockner and Antia 1986) occurred in high abundance ($>6 \times 10^4$ cells·mL⁻¹) in July and declined to densities of less than 3×10^4 cells·mL⁻¹ by the end of August. These organisms were present primarily as small colonies ranging from 5 to 95 cells in size.

The zooplankton community of the Little Lagoon was highly dynamic during this study (Fig. 1b). *Bosmina longirostris* was the most numerous taxon at the beginning of the study but was outnumbered by *C. reticulata* and cyclopoids for the final three samplings. *Ceriodaphnia reticulata* densities increased to a peak of almost 16 ind·L⁻¹ on August 22 and then declined to less than 4 ind·L⁻¹ by August 30. *Daphnia rosea* densities remained extremely low (<0.5 ind·L⁻¹), probably as a result of the high densities of planktivorous fish present in the Little Lagoon during this period.

Phytoplankton Community Responses in Enclosures

Chlorophyll *a* concentrations in the enclosures exhibited highly significant treatment responses (Fig. 2). As predicted by the cascading trophic interactions hypothesis (Carpenter et al.

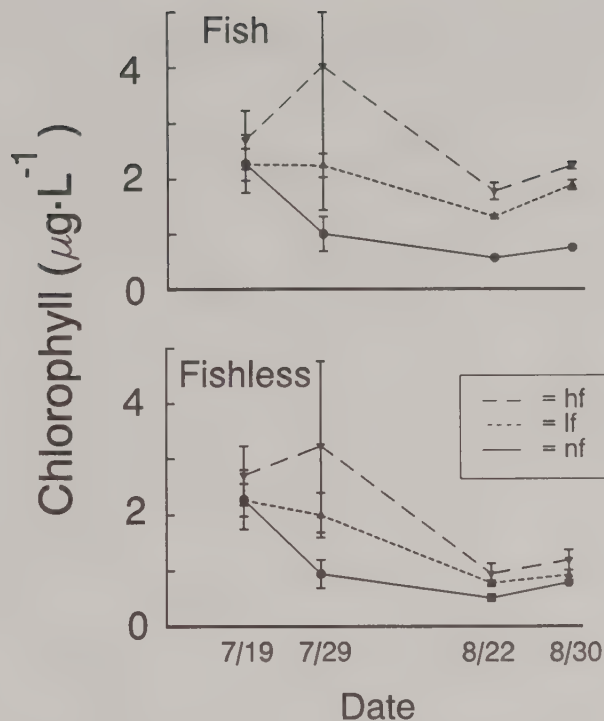


FIG. 2. Chlorophyll *a* concentrations in "fish" and "fishless" compartments of the enclosures. Means ($\pm$ standard error) of replicate enclosures are represented. nf = no-fish treatment, lf = low-fish treatment, hf = high-fish treatment.

1985), chlorophyll *a* concentrations were consistently highest in the "fish" compartments of enclosures treated with the high-fish density and lowest in the control (no-fish) enclosures (χ^2 , $p < 0.03$) (Fig. 2). Of particular interest to this study is the fact that the same trends were observed in the "fishless" compartments of the enclosures. Specifically, in the absence of direct predation, the fishless compartments of the high-fish enclosures supported the highest chlorophyll *a* concentrations, the low-fish enclosures supported intermediate concentrations, and the no-fish enclosures supported the lowest (χ^2 , $p < 0.03$) (Fig. 2). Total phytoplankton biomass exhibited definite positive responses to the fish treatments in both the "fish" and "fishless" compartments (χ^2 , $p < 0.03$) (Fig. 3).

The grazable component of the phytoplankton community in the "fish" compartments (almost exclusively composed of *Chlamydomonas* spp. and *Cryptomonas* spp.) was maintained at highest biomass in the high-fish enclosures and at lowest biomass in the no-fish enclosures (χ^2 , $p < 0.03$) (Fig. 3). In the "fishless" compartments, the highest biomass of the grazable component was once again in the high-fish enclosures (χ^2 , $p < 0.03$), but no significant difference was detected between the no-fish and the low-fish treatments (χ^2 , $p > 0.20$) (Fig. 3).

The ungrazable component of the phytoplankton community in the enclosures was composed exclusively of colonial *Dinobryon* spp. in all enclosures. No significant differences in biomass of this component were detected between the control and low-fish treatment enclosures ("fish" and "fishless" compartments) (Friedman's, $p > 0.20$). Both the "fish" and the "fishless" compartments of the high-fish treatment enclosures developed substantial (χ^2 , $p < 0.03$) blooms of this alga by August 22 when it comprised up to 47% of the phytoplankton

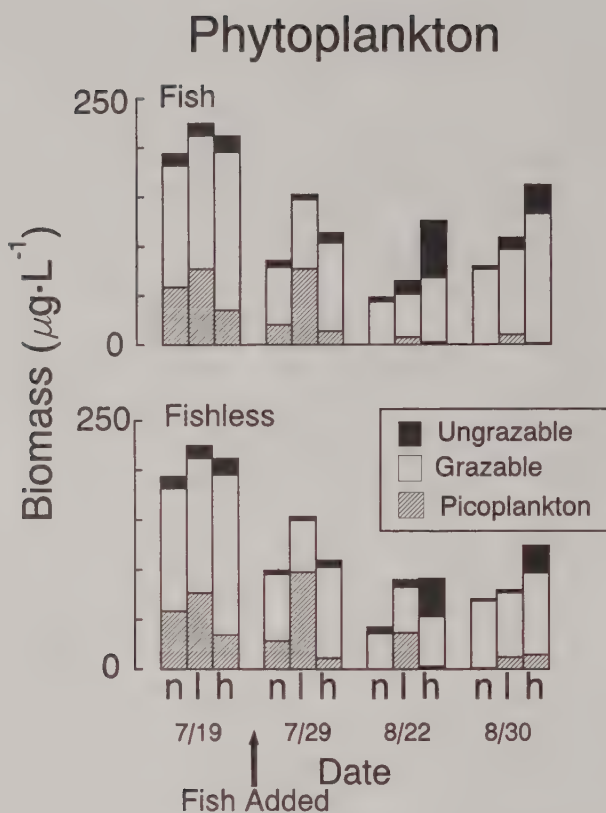


FIG. 3. Phytoplankton dry biomass in "fish" and "fishless" compartments of the enclosures. Biomass was estimated for three fractions of phytoplankton based on their expected grazability. Means of replicate enclosures are represented. n = no-fish treatment, l = low-fish treatment, h = high-fish treatment.

community biomass (Fig. 3). On August 30, this component still comprised as much as 25% of the phytoplankton community biomass in both compartments of all high-fish enclosures.

Picoplankton densities declined over the course of the study similarly to the densities observed in the Little Lagoon. Regardless of this trend, picoplankton were maintained at highest densities in the low-fish enclosure (χ^2 , $p < 0.03$) ("fish" and "fishless" compartments) (Fig. 3). No effect was detected in the high-fish treatments.

Periphytic chlorophyll *a* sampled from the inside of the enclosure walls at the end of the experiment exhibited no significant treatment response in either the "fish" or the "fishless" compartments (Kruskal-Wallis, $p = 0.17$).

Zooplankton Community Responses in Enclosures

Calculation of total zooplankton community biomass in the "fish" compartments showed that significant (χ^2 , $p < 0.03$) differences existed between the controls, the low-fish treatments, and the high-fish treatments, with total biomass decreasing in that order (Fig. 4). In the "fishless" compartments, the low-fish enclosures supported the highest biomass (χ^2 , $p < 0.03$) over the course of the study, while the controls and high-fish enclosures supported equivalent amounts of zooplankton biomass (Fig. 4).

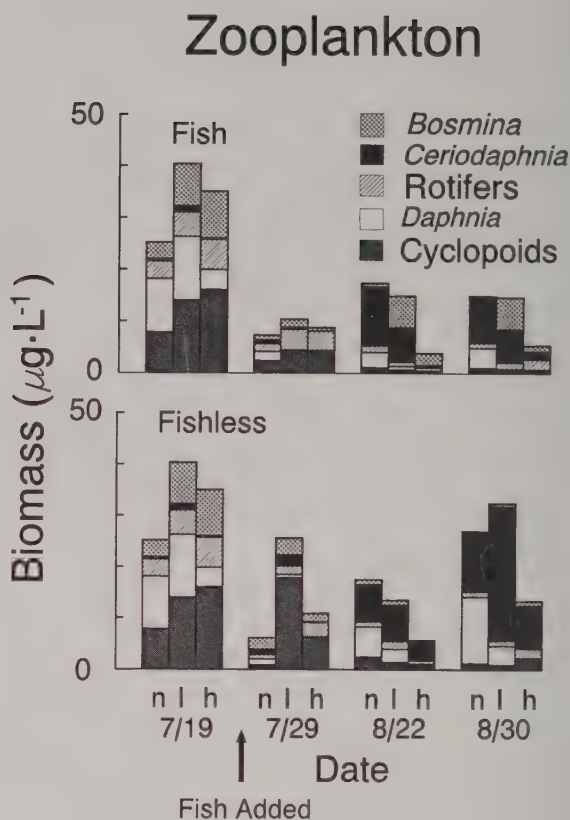


FIG. 4. Zooplankton dry biomass in "fish" and "fishless" compartments of enclosures. Means of replicate enclosures are represented. Treatment abbreviations as in Fig. 3.

The zooplankton in the "fish" compartments of enclosures were greatly impacted by the sockeye fry present there (Fig. 4). *Daphnia* were virtually eliminated in the low-fish and high-fish treatments but remained at significantly higher densities in the control enclosures (χ^2 , $p < 0.03$). *Ceriodaphnia* did not change as dramatically as *Daphnia*. *Ceriodaphnia* in the no-fish and the low-fish treatment enclosures remained at similar population sizes throughout the study but were significantly (χ^2 , $p < 0.03$) larger than the populations in the high-fish treatment enclosures. All other taxonomic groups exhibited no responses to the fish treatments in the "fish" compartments.

Zooplankton in "fishless" compartments of enclosures also responded to fish treatments (Fig. 4). *Ceriodaphnia*, *Daphnia*, and rotifers in low-fish treatment enclosures showed significant (χ^2 , $p < 0.03$) increases over the controls, while *Bosmina* and cyclopoid copepod populations were equivalent to those in controls. The zooplankton communities in the high-fish enclosures responded very differently from those in the low-fish enclosures. *Daphnia* were much scarcer than in the control enclosures (χ^2 , $p < 0.03$), while all other taxonomic groups remained at population sizes equivalent to the controls (Fig. 4). The decline of *Daphnia* populations coincided with the blooms of the colonial alga *Dinobryon* in the high-fish enclosures (Fig. 3).

P Excretion Rates

Comparison of P excretion rates in the enclosures showed that the fish contributed approximately seven times more than

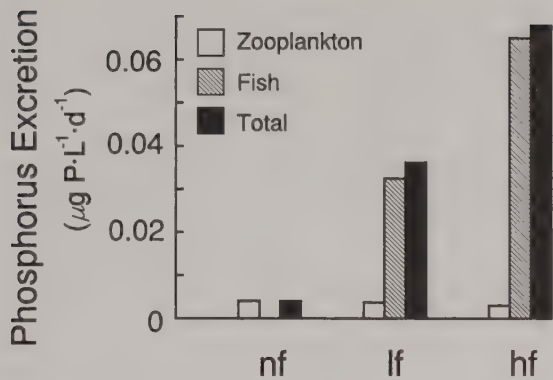


FIG. 5. Estimated rates of P excretion by zooplankton and sockeye fry on August 22. Rates represent excretion of soluble reactive P by zooplankton and fish for each entire enclosure. Means of replicate enclosures are represented. Treatment abbreviations as in Fig. 2.

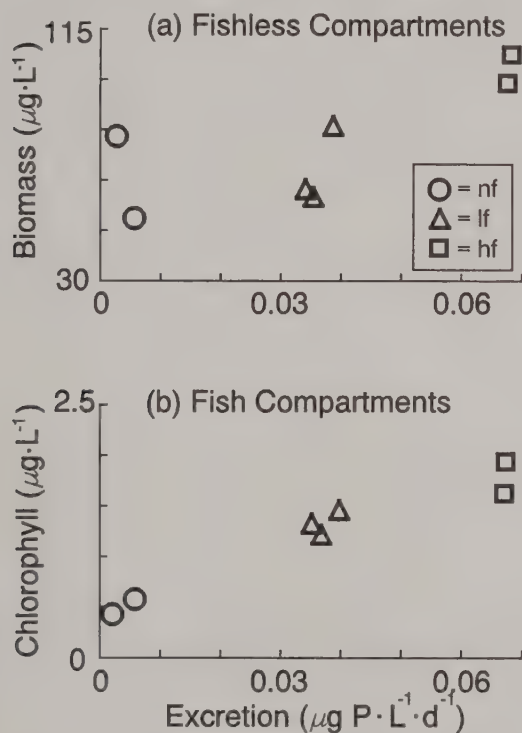


FIG. 6. (a) Regression of total plankton (zooplankton + phytoplankton) dry biomass on combined (fish + zooplankton) P excretion rate in the "fishless" compartments of enclosures. The model to describe this relationship is $TPB = 53.6 + 647.8(ER)$ where TPB = total plankton dry biomass and ER = total excretion rate ($p = 0.05$, $r^2 = 0.55$). (b) Regression of chlorophyll *a* concentration on combined P excretion rates for the "fish" compartments of enclosures. The model to describe this relationship is $CHL = 0.494 + 20.2(ER)$ where CHL = chlorophyll *a* concentration ($p < 0.001$, $r^2 = 0.95$). Estimates for individual enclosures are represented. Treatment abbreviations as in Fig. 2.

zooplankton in the low-fish treatments and approximately 28 times more in the high-fish treatments (Fig. 5). This difference in magnitude of P supply is due to both higher fish biomass in

the high-fish treatments and a marked decline in zooplankton biomass in those enclosures. Regressing total plankton biomass (zooplankton dry mass + phytoplankton dry mass) in the "fishless" compartments against the estimated combined P excretion rate for each of the enclosures showed that a significant relationship (F test, $p = 0.05$) existed between these two variables (Fig. 6a). Phosphorus excretion rates by fish and zooplankton accounted for 55% of the variation in total plankton biomass in the "fishless" compartments of the enclosures.

Regressing chlorophyll *a* concentration (as an index of phytoplankton biomass) in the "fish" compartments against excretion rates showed a highly significant (F test, $p < 0.001$) relationship between these two variables in the enclosures (Fig. 6b). Phosphorus excretion rates could explain 95% of the variance in chlorophyll *a* concentration in the "fish" compartments of the enclosures. No significant relationship was detected between community grazing rate and chlorophyll *a* concentration (F test, $p = 0.56$). Addition of community grazing rate as an independent variable to a multiple regression model (with excretion rate) did not improve its ability to predict chlorophyll *a* concentrations.

Discussion

The results of this experiment show that planktivorous fish can directly affect phytoplankton in ways that are distinct from those caused by predator control of the herbivore community. Evaluation of the potential of fish excreta as a source of P to phytoplankton shows that this is an important mechanism through which planktivorous fish may enhance algal production. Although the effects of decreased grazing pressure due to predator control of the herbivore community must act commensurately with the effects of nutrient regeneration by fish to increase algal production, its universal importance may be overstated (Carpenter et al. 1985). For instance, the results of this experiment show that 95% of the variation in chlorophyll *a* concentrations between fish treatments could be predicted by P excretion rates alone.

The fact that increases in total plankton biomass were detected in response to the fish treatments in the "fishless" compartments of the enclosures verifies that direct fish effects on phytoplankton were important in this study. However, because the kinetics of P cycling are so rapid, this analysis may be a conservative estimate of the magnitude of the effects of fish excretion on phytoplankton. Any nutrients regenerated in a "fish" compartment must have remained in soluble form long enough to diffuse to a "fishless" compartment if they were to enhance algal production there. Algae will deplete small quantities of soluble reactive P (the form of P fish excrete; Braband et al. 1990) from dilute solution within a time scale of 1–2 h (Lehman and Sandgren 1982). Turbulent diffusion would have thoroughly mixed my enclosures within a time scale of 1–5 min (calculation based on estimated average turbulent diffusion rate of $0.3\text{--}1.0\text{ cm}\cdot\text{s}^{-1}$ (Noble 1961) and assuming that Nitex mesh does not substantially impede water movement through it). Therefore, there was probably ample opportunity for most of the P excreted by fish to be mixed throughout the enclosures before it was assimilated into algae. The goodness of fit of the regression of chlorophyll *a* concentration versus P excretion in the "fish" compartments is probably a more qualitative assessment of the direct impacts of fish excretion on phytoplankton.

The ability of P excretion rates to predict 95% of the variation in chlorophyll *a* concentration suggests that predator control of the herbivore community had practically no effect on phyto-

TABLE 1. Average lengths and standard deviations of the zooplankton in the "fish" compartments of the enclosures and the Little Lagoon at the end of the experiment. A matrix of the results from a pairwise multiple comparison test (Tukey test) shows which pairs of mean lengths were not different (ND) or significantly different (D) from each other with 95% confidence.

Treatment	Mean length (mm)		SD	
No-fish	0.842		0.120	
Low-fish	0.467		0.053	
High-fish	0.395		0.080	
Little Lagoon	0.498		0.016	

	No-fish	Low-fish	High-fish	Little Lagoon
No-fish	—			
Low-fish	D	—		
High-fish	D	D	—	
Little Lagoon	D	ND	D	—

plankton biomass in this experiment. Several factors probably contributed to this result. For example, the blooms of *Dinobryon* that developed in the high-fish treatment enclosures were probably not strongly affected by herbivores due to its poor edibility (W. E. Neill, University of British Columbia, pers. comm.). In fact, herbivore consumption of the grazable algae may have facilitated the bloom of *Dinobryon* by alleviating the competition for a limiting resource such as P. The absence of very large cladoceran grazers (*D. rosea*, maximum length = 1.5 mm in enclosures) in the zooplankton potentially limited the effectiveness of the herbivore community in substantially controlling phytoplankton biomass (Knoechel and Holtby 1986). The composition and size structure of the zooplankton community were already affected by planktivory in the Little Lagoon before being added to the enclosures.

The densities of fish present in a system, whether it be a natural one or an experimental mesocosm, will affect the magnitude and the qualitative nature of any phytoplankton response to them. For instance, different levels of fish predation on zooplankton will affect the grazing pressure on algae, influencing both the standing stock and the edibility of the algal community. Differences in the N:P ratios from animal excreta may also be affected by the ratio of zooplankton biomass to fish biomass (Carpenter and Kitchell 1984; Kraft 1991). Although fish density data for the Little Lagoon are unavailable, I am confident that the fish densities I used as treatments were within the range of natural fish densities in the lake. Evidence for this is provided by a comparison of the average lengths of zooplankton present in the "fish" compartments of the enclosures with those in the lake. The average length of the zooplankton in the lake was not significantly different from that of the zooplankton in the low-fish enclosures (Table 1). Significant differences were detected between the lake and all other enclosures. Using the inverse of average zooplankton length as a surrogate for the magnitude of predation pressure by fish on zooplankton (Mills and Schiavone 1982) suggests that the density of fish present in the low-fish enclosures was a reasonable approximation of that in the lake. The high-fish treatment may have therefore created a greater than natural fish density.

Fishes that feed obligately on zooplankton cannot excrete more nutrients than zooplankton. However, most fishes supplement their diets with benthic prey (Scott and Crossman 1985) which may allow some lakes to be characterized by fish communities whose biomass exceeds zooplankton community biomass (Kitchell et al. 1975). In lakes such as these, fish have

the potential to excrete more nutrients than zooplankton communities (Carpenter et al. 1992) at long time scales.

My experiment demonstrated that fish result in greater accumulations of algae which, in turn, can be attributed to greater availability of a limiting nutrient such as P. Several possible mechanisms may account for this increased availability of P to algae. Decaying fish carcasses and fish that are losing mass may regenerate substantial quantities of nutrients (Threlkeld 1987, 1988). However, I know that all of the experimental fish were alive for the entire experiment, and have assumed their mass to have remained constant. Therefore, I believe that the importance of P regeneration by these mechanisms to be unimportant in this experiment. Fish may increase the rate of mineralization of nutrients from the sediments by consuming benthos and/or by resuspending surface sediments, thereby allowing for greater nutrient release (Lamarra 1975). Sockeye salmon fry are generally thought to feed obligately in the pelagic zone of lakes. It is possible, however, that they feed on benthos in the Little Lagoon and in the enclosures because the whole system is essentially littoral zone. I did not monitor the benthos in the enclosures or the Little Lagoon and therefore do not know whether fish consumed them. Fish consumption of benthos may have provided the planktonic algae in this system with an important source of nutrients normally not available to them. Although nutrient recycling within the benthos may mineralize significant quantities of nutrients (Boers et al. 1991), whether it is available to pelagic algae in the absence of fish is debatable. Braband et al. (1990) showed that fish consumption of littoral zone P and its subsequent excretion in the pelagia provides an important nutrient link between these two habitats and thereby contributes to eutrophication in lakes. Similar analyses of nutrient/fish interactions on coral reefs have shown that fish can act as an important transport vector of nutrients between spatially segregated habitats within ecosystems (Meyer et al. 1983; Meyer and Schultz 1985).

Although nutrient inputs and recycling ultimately determine the range of total organic matter a system can support (Quiros 1990), other factors determine how nutrients are distributed among various trophic levels of a food web. For example, Boers et al. (1991) argued that P availability to phytoplankton depends strongly on the sedimentation rate of total P and its interaction with remineralization processes in a system.

Planktivorous fish have also been shown to cause increased P recycling rates in experimental mesocosms (Mazumder et al. 1988). Fish acting both as a direct source of nutrients through excretion and causing an indirect increase in the excretion rate of nutrients by small-bodied zooplankton (Carpenter and Kitchell 1984; Vanni and Findlay 1990) may account for these increased nutrient cycling rates. Although I did not measure nutrient recycling rates, my results strongly suggest that increases must have been due to direct fish excretion and not to indirect zooplankton effects. Increased zooplankton excretion was probably unimportant because it contributed a small fraction of the total excreted P to the enclosures, and because zooplankton biomass was negatively affected by the fish treatments.

The hypothesis of cascading trophic interactions predicts how organic matter is distributed among different trophic levels in aquatic ecosystems (Paine 1980; Carpenter and Kitchell 1984). The strength of linkages between the various trophic levels of ecosystems determines whether predators at the top of food webs have detectable "cascading" effects on lower trophic levels. Although linkages between nonadjacent trophic levels are often transduced via an intermediate trophic level (e.g.

planktivorous fish can affect phytoplankton biomass by control of the zooplankton community), direct linkages between such nonadjacent trophic levels can also be important. This study has shown that nutrient excretion by fish can directly enhance algal production that has important implications in analyzing the mechanisms by which cascading trophic interactions operate in aquatic systems (Carpenter and Kitchell 1984; Vanni and Findlay 1990). The discrepancies between the findings of this and similar studies (e.g. Braband et al. 1990; Reinertsen et al. 1990; Vanni and Findlay 1990; Kraft 1991) and other less direct and specifically focused studies of fish effects on lower trophic levels (e.g. Kitchell et al. 1979; Nakashima and Leggett 1980) suggest that the nutrient effects of fish on phytoplankton are very complex and may have fundamentally different implications in different lake ecosystems. The direct nutrient effects of fish on phytoplankton have certainly been underestimated in previous analyses of limnetic food webs (e.g. Kitchell et al. 1975; Nakashima and Leggett 1980).

The direct effect of fish on phytoplankton will vary in proportion to fish densities within a habitat. Their mobility can increase or decrease that effect through migrations (e.g. Braband et al. 1990) and aggregation. These dynamics operate at different spatial and temporal scales. Total effects may vary with changes in interannual population density (e.g. recruitment success) and fish community composition (e.g. percent planktivores; Leavitt et al. 1989). Intraannual effects vary with the annual temperature cycle as it regulates the rates of predation and nutrient excretion. Short-term effects may also vary with ontogenic changes in prey selection, and the diel onshore-offshore (Braband et al. 1990) or vertical migrations (Levy 1990) that evidence dynamic predator-prey interactions (Kerfoot and Sih 1987). Population and behavioural responses of fishes therefore become fundamentally important independent variables that influence algal dynamics in lotic systems.

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Acidification Effects on the Algal–Zooplankton Interface

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An in situ mesocosm experiment was done to assess the impacts of acidification on algal and zooplankton biomass and C assimilation, and on the efficiency of energy transfer in the grazing food chain. Triplicate mesocosms were left untreated (pH 8.5), or were acidified to pH 6.5, 5.5, or 4.5 with H_2SO_4 over 9 d. Algal biomass was reduced at pH 6.5 and lower, but showed no further decline across the gradient of pH 6.5–4.5. Algal C assimilation rates consistently declined with decreasing pH, reflecting a shift in dominance to larger, less productive cells at pH 5.5 and 4.5. Zooplankton biomass and productivity also declined with decreasing pH, as nearly all crustacean herbivores became extinct. In the low-pH treatments, only cyclopoids and rotifers persisted. Overall, there were significant reductions in the ratios of zooplankton/algal biomass and zooplankton/algal C assimilation (ecological transfer efficiency) with declining pH. The latter was a result of reduced grazer biomass, rather than reduced grazing efficiency; the mean zooplankton *P/B* ratio at pH 4.5 exceeded that measured in the higher pH treatments.

Une expérience a été effectuée sur des mésocosmes en-situ pour évaluer les effets de l'acidification sur la biomasse des algues et du zooplancton et de l'assimilation de C, ainsi que sur l'efficacité du transfert d'énergie dans la chaîne alimentaire de broutage. Des mésocosmes triples ont été laissés non traités (pH 8,5), ou ont été acidifiés à pH 6,5, 5,5 ou 4,5 avec du H_2SO_4 durant 9 jours. La biomasse alguaire a été réduite à un pH 6,5 et inférieur, mais n'a montré aucune diminution dans le gradient de pH 6,5 à 4,5. Les taux d'assimilation du C par les algues ont baissé régulièrement avec la baisse du pH, ce qui indique un transfert de dominance à des cellules plus grosses moins productive aux pH 5,5 et 4,5. La biomasse et la productivité du zooplancton ont également diminué avec la baisse du pH, avec la disparition presque totale des crustacés herbivores. Dans les traitements à pH inférieur, seuls les cyclopoïdes et les rotifères ont survécu. Dans l'ensemble, il y a eu des réductions significatives des ratios de biomasse zooplancton/algues et de l'assimilation carbonique zooplancton/algues (efficacité du transfert écologique) avec la baisse du pH. Cette dernière était due à une réduction de la biomasse des brouteurs, plutôt qu'à la diminution de l'efficacité du broutage; le ratio moyen de *P/B* du zooplancton à pH 4,5 dépassait celui mesuré dans les traitements à pH plus élevé.

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Acidification results in pronounced changes in lake ecosystem structure and function (Schindler 1990). It causes a succession involving all trophic levels, wherein acid-sensitive species become extinct, and acid-tolerant species increase in abundance. Plankton responses to acidification have been studied for several decades, and are now well documented. The total number of zooplankton species is reduced with declining pH (Sprules 1975; Confer et al. 1983) as is overall food web complexity (Havens 1991a). Large herbivores like *Daphnia galeata mendotae*, *D. pulex*, *D. pulicaria*, and *D. retrocurva* are particularly sensitive and are absent from most lakes of pH <5.5 (Carter et al. 1986; Malley and Chang 1986; Keller et al. 1990). In contrast, the small-bodied herbivores *Bosmina longirostris*, *Diaptomus minutus*, and *Keratella taurocephala* increase in abundance with acidification, and are typically the extreme dominants in acid lakes (Sprules 1975; Yan and Strus 1980; Havens and DeCosta 1985; Yan and Geilinger 1985). In acid lakes having low metal levels, the cladoceran *Holopedium gibberum* is often a codominant (Keller and Yan 1991). Overall, there is a significant reduction in mean herbivore body size with declining pH (Pinel-Alloul et al. 1990; Tessier and Horwitz 1990). Coincident with the zooplankton succession, there are pronounced changes in the phytoplankton community. Diatoms and chrysophytes, which dominate in unpolluted soft waters, decline during acidification, while dinoflagellates increase (Yan 1979; Stokes and Yung 1986; Findlay

and Kasian 1990). In acid lakes, dinoflagellates, especially *Peridinium inconspicuum*, account for large proportions of total algal biomass (Yan 1979; Avalos 1981; Havens and DeCosta 1985; Findlay and Kasian 1986). Overall, there is an increase in mean algal cell size with declining pH (Schindler 1990; Havens and Heath 1991).

Yan and Strus (1980) hypothesized that such changes in the zooplankton and phytoplankton might result in a bottleneck to energy flow in the grazing food chain. Small-bodied herbivores like *Bosmina* do not exploit the large algal cells available to daphnids (Burns 1968), and it has been demonstrated that the *Bosmina* and *Keratella* populations in acid lakes do not feed on *P. inconspicuum*, even when it is the dominant alga (Yan and Strus 1980; Havens and DeCosta 1985). The low ratio of zooplankton/algal biomass observed in acid lakes (Olofsson et al. 1988; Yan and Welbourn 1990) is evidence that energy flow to higher trophic levels is suppressed by this bottleneck. However, no studies have quantified the effect.

The objective of this study was to assess the impacts of acidification on algal and zooplankton biomass and C assimilation, and on the efficiency of energy transfer in the grazing food chain. The approach was to acidify lake water inside replicated in situ mesocosms from pH 8.5 (control) to pH 6.5, 5.5, and 4.5 over a 10-d period. Initial and final determinations were made of algal and zooplankton biomass and taxonomic composition, and algal and zooplankton C assimilation rates. The

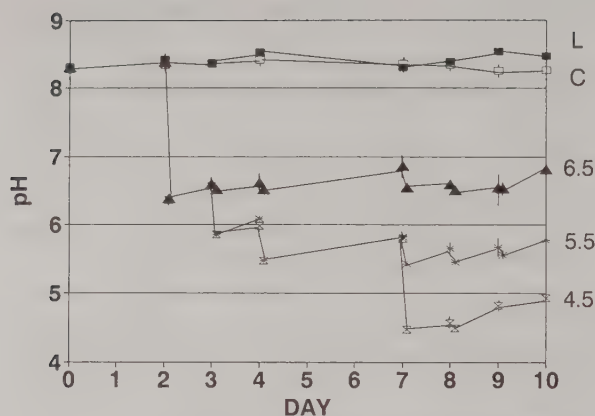


FIG. 1. pH in the lake and treatments during the experiment. Vertical bars are ± 1 standard error.

ratio of zooplankton/algal C assimilation in each treatment was determined as an index of ecological transfer efficiency.

Materials and Methods

Study Site

The experiment was done at East Twin Lake, OH, USA (latitude $41^{\circ}11'$, longitude $81^{\circ}20'$, altitude 350 m, area 27 ha, maximum depth 12 m, mean depth 5 m). The lake is well buffered (pH 8–9, alkalinity $1.6 \text{ meq}\cdot\text{L}^{-1}$) and oligomesotrophic based on summer 1991 chlorophyll and total P concentrations (both $<5 \mu\text{g}\cdot\text{L}^{-1}$). The summer phytoplankton is dominated by nanoflagellates, cryptomonads, dinoflagellates, cyanobacteria, and chrysophytes. *Daphnia galeata mendotae*, *D. retrocurva*, *Eubosmina coregoni*, *Diaphanosoma birgii*, *Skistodiaptomus oregonensis*, and *Keratella cochlearis* are the numerically dominant herbivores. East Twin Lake was the site of several previous studies of acid and Al impacts on the plankton (Havens 1990; Havens and Heath 1989, 1990, 1991). It is a suitable control for acidification studies; the plankton is similar to that of acid-sensitive soft waters and, in the previous experiments, communities which developed in acidified mesocosms closely resembled those seen in precipitation-acidified lakes.

Experimental Design

On 9 August 1991, 12 transparent polyethylene bags were filled with 60 L of epilimnetic water passed through a 200- μm nylon mesh. The water contained lake densities of algae, nauplii, and rotifers, but was nearly free of adult crustaceans. Immediately after filling each bag, zooplankton were collected from the 6-m water column with a vertical tow of a 200- μm plankton net (total volume sampled assuming 100% efficiency = 57 L). The contents of the net were released into each bag, resulting in a total zooplankton biomass that closely approximated the water column mean. This procedure was necessary because the daphnids and diaptomids in East Twin Lake undergo nocturnal migration. The bags were tied closed with nylon line, attached to weights in groups of four, and suspended at a depth of 2 m (midepilimnion) from surface floats. The bag and float design is similar to that described in Porter (1972).

Acidification began 2 d after the bags were filled. The pH was determined in situ using an Orion meter and Ross combination electrode (Metcalf 1987). After measuring initial pH

(days 0–2), nine bags were spiked with reagent-grade H_2SO_4 , to reduce pH to 6.5. The remaining three bags were untreated controls. On day 3, six of the acidified bags received a further acid addition, reducing pH to roughly 5.5. On day 7, a final pH adjustment was done in three of the pH 5.5 bags, reducing pH to roughly 4.5. The end result was three bags each at roughly pH 8.5 (control), 6.5, 5.5, and 4.5 after 7 d of adjustment (Fig. 1). Additional acid additions were required during the experiment in order to maintain pH at the desired levels.

Sampling Procedures

The bags and adjacent lake water were sampled on days 0 (initial) and 9 (final). Immediately after opening each bag, the contents were mixed with an oar and phytoplankton were collected in Whirl-pak bags. Lugols solution (2 mL per 100 mL of sample) was added, and the samples were stored in a dark box until enumeration. Periphyton were not sampled, as growth was minimal during the short experiment. Zooplankton were collected by immersing a 4-L plastic carboy into each bag, and then pouring the contents into a plastic cup with a 40- μm -mesh side window. Retained animals were rinsed into Whirl-paks and preserved with 4% (final concentration) ice-cold formalin-sucrose (Prepas 1978). On day 0, zooplankton from the lake were also returned to the laboratory live for determination of crustacean dry weights.

Plankton Enumeration and Biomass Determination

Phytoplankton samples were settled for 48 h in 10-mL chambers, and enumerated using a Zeiss Axiovert microscope. For each sample, replicate strips were scanned at $\times 640$ magnification, until at least 200 cells were counted. The entire chamber base was also scanned at $\times 100$ for enumeration of large, rare species. Population densities (cells per litre) were determined using the equation given in Lund et al. (1958). At least 20 cells were also measured for each algal species, and mean cell volumes (cubic micrometres per cell) determined by approximating shapes to regular geometric solids. Cell volumes were converted to biomass units (micrograms C per cell) using Strathmann's (1967) equation: $\log C = -0.460 + 0.866 (\log V)$, where C is carbon (picograms) and V is volume (cubic micrometres). For diatoms, which have a lower C content per unit volume, the equation $\log C = -0.422 + 0.758 (\log V)$ (Strathmann 1967) was used. The population biomass of each algal species (micrograms C per litre) was determined by multiplying density times cell biomass. Total algal biomass was determined by summation of the individual species biomass values.

Zooplankton samples were concentrated to a known volume, using the plastic cup with a 40- μm -mesh side window. Aliquots (5 mL) were settled for roughly 30 min in cylindrical chambers, and the entire chamber base scanned at $\times 80$ for enumeration of rotifers and nauplii. The procedure was repeated until at least 200 animals were counted. A minimum of 20 individuals of each rotifer species, as well as 20 calanoid and cyclopoid nauplii, were measured at $\times 80$ and volumes determined as for the algae. Volumes were converted to fresh weights by assuming unit density ($1 \text{ g}\cdot\text{cm}^{-3}$); fresh weights were converted to dry weights according to the equation $\text{dw} = \text{fw}\cdot 0.1$ (Pace and Orcutt 1981). Dry weights were then converted to C units (micrograms C per animal) according to the equation $\mu\text{g C} = \mu\text{g dw}\cdot 0.48$ (Andersen and Hessen 1991).

Cladocerans, adult copepods, and copepodids were counted by scanning the entire sample volume under a dissecting micro-

scope at $\times 20$ magnification. Three groups of 10 randomly chosen live individuals of each crustacean species (and copepodid stages for the copepods) were taken from the unpreserved lake sample, dried at 80°C for 48 h, and weighed to $\pm 0.5\ \mu\text{g}$ with a Sartorius S4 ultramicrobalance. Mean dry weights (micrograms per animal) were converted to micrograms C per animal as for the rotifers.

Zooplankton (crustacean and rotifer) population densities (individuals per litre) were determined from counts assuming 100% sampling efficiency. Densities were converted to population biomass units (micrograms C per litre) by multiplying density by biomass for each species and developmental stage. Total zooplankton biomass was calculated by summation of the individual population biomass values.

Algal C Assimilation

Relative algal C assimilation rates were determined on days 0 and 9 using the ^{14}C light and dark bottle technique under standardized illumination conditions. Unfiltered water was collected from each bag and the adjacent lake in duplicate light and dark acid-rinsed, autoclaved BOD bottles. Water was also collected in HCl-rinsed amber plastic bottles for determination of alkalinity. All bottles were stored in a covered box, and returned to the laboratory within 1 h of sampling. Each BOD bottle was inoculated with $5\ \mu\text{Ci}$ of $\text{NaH}^{14}\text{CO}_3$ ($1\ \mu\text{Ci} = 37\ \text{kBq}$), placed under a fluorescent light bank, and incubated at $20\text{--}21^{\circ}\text{C}$ for 2 h. Triplicate vials containing 5 mL of buffered scintillation cocktail were also inoculated with $\text{NaH}^{14}\text{CO}_3$ ($100\ \mu\text{L}$) for determination of available ^{14}C . During the incubation, pH and total alkalinities were determined using the Orion/Ross system, and the H_2SO_4 titration procedure of Environment Canada (1979). Available ^{12}C concentrations (micrograms per litre) were determined from the pH, temperature, and alkalinity data, according to Vollenweider (1974). At 2 h, each BOD bottle was mixed by inversion, and a 5-mL aliquot removed and filtered through a nucleopore $0.2\text{-}\mu\text{m}$ filter under a vacuum of $0.1\ \text{atm}$ ($10.13\ \text{kPa}$). The exact time was recorded, and the filter was inverted over a beaker containing concentrated HCl. After fuming for 5 min (to drive off unassimilated ^{14}C), the filter was placed into a vial with 5 mL of scintillation cocktail. Particulate ^{14}C uptake rates were determined using a Beckman liquid scintillation counter, correcting for background and color quenching. Rates of ^{12}C uptake (micrograms C per litre per hour) were determined from the ^{14}C available, ^{12}C available, ^{14}C uptake, and incubation time, according to the equation given in Vollenweider (1974). Photosynthetic C assimilation rates (PCA) were determined by subtracting the mean dark bottle rates from the light bottle rates.

Zooplankton C Assimilation

Zooplankton C assimilation rates in the bags and lake were measured on days 1 and 10, using the in situ procedure of Bogdan and McNaught (1975), as modified by Havens (1991b). In this procedure, the natural algal assemblage is used as the radiolabelled food for determining grazing or assimilation rates. On days 0 and 9, water from each bag and the lake was poured through a $40\text{-}\mu\text{m}$ mesh (to remove zooplankton) into 1-L HCl-rinsed autoclaved plastic bottles, and spiked with $100\ \mu\text{Ci}$ of $\text{NaH}^{14}\text{CO}_3$. The bottles were capped, placed into the bags, and incubated in situ for 48 h to allow for labelling of the algal cells. At roughly 09:00 on days 1 and 10, the following procedure was performed in each bag. The radiolabelled algae were retrieved and placed into a dark box. A 4-L volume of water

was collected from the bag with the zooplankton sampler, and poured gently into a $5 \times 30\ \text{cm}$ plastic cylinder with a $40\text{-}\mu\text{m}$ -mesh base. During the process, the base of this "grazing chamber" was submerged in the bag. The chamber was raised from the water and placed into the bottle of radiolabelled algae, which entered through the mesh base. After 5 min (less than the gut passage time of the dominant herbivores, as determined by preliminary observations of animals feeding on fluorescent microspheres), the chamber was lifted from the bottle, retaining zooplankton but allowing algae to exit. The animals were gently rinsed with filtered lake water, and the chamber immersed into a beaker of unlabelled water from the bag for 30 min. During that time, the grazers cleared their guts of radiolabelled food (based on preliminary estimates of gut passage time using fluorescent microspheres), such that activity remaining in their bodies approximated the amount of ^{14}C assimilated into tissues from the 5-min slug of radioactive food. At 30 min, the grazing chamber was removed, and the animals were immediately rinsed onto a $0.45\text{-}\mu\text{m}$ Nucleopore filter. After evacuating the water, the filter was placed into a vial with 0.5 mL of NCS tissue solubilizer. The vial was incubated at 80°C for 24 h, inoculated with two drops of glacial acetic acid and 5 mL of scintillation cocktail, and counted on the liquid scintillation counter with corrections for background and color quenching. A sample from each bottle was also counted for determination of the feeding suspension activity. It was collected during the 5-min grazing period, by taking 5 mL of suspension from the bottle, collecting the algae on a $0.2\text{-}\mu\text{m}$ Nucleopore filter, fuming for 5 min over concentrated HCl, and then placing the filter into a vial of cocktail. Because both the grazers and algae were placed into scintillation vials within minutes of collection, tracer losses were considered to be negligible in these experiments. Carbon assimilation rates by the zooplankton community (ZCA) in each bag were determined as

$$\begin{aligned} \mu\text{g C assimilated} \cdot \text{L}^{-1} \cdot \text{h}^{-1} \\ = \frac{(\text{dpm} \cdot \text{L of grazers}^{-1}) \cdot t \cdot \text{C}}{(\text{dpm} \cdot \text{mL of feeding suspension}^{-1})} \end{aligned}$$

where t is a temporal correction factor (per hour) of 12 to account for the 5-min grazing time and C is the calculated carbon content (micrograms C per millilitre) of the $<20\ \mu\text{m}$ algal size fraction in each bag. The $20\text{-}\mu\text{m}$ cutoff was intended to separate "edible" from "inedible" algae in the treatments. In the lake and control, where cladoceran, copepod, and rotiferan herbivores were abundant, the $<20\ \mu\text{m}$ fraction was composed of nanoflagellates, *Chlamydomonas* spp., *Rhodomonas minuta*, *Selenastrum minutum*, and *Cryptomonas erosa*. The $>20\ \mu\text{m}$ fraction was composed of *Dictyosphaerum ehrenbergianum*, *Anabaena* sp., *Ceratium hirundinella*, and *Gymnodinium* sp. In the pH 4.5 treatment, where rotifers were the herbivores, the $<20\ \mu\text{m}$ fraction was composed of nanoflagellates and *Chlamydomonas* sp. The $>20\ \mu\text{m}$ fraction was composed of *P. inconspicuus*, *Carteria klebsii*, *Oocystis* sp., *Scenedesmus bijuga*, and *Schroederia* sp.

Ecological Transfer Efficiency

For each bag and the lake, ecological transfer efficiencies were calculated on days 0–2 and 9–10. The efficiencies reflected the portion of C assimilated by phytoplankton which was subsequently assimilated by zooplankton, and were calculated as $\text{ZCA}/\text{PCA} \cdot 100\%$.

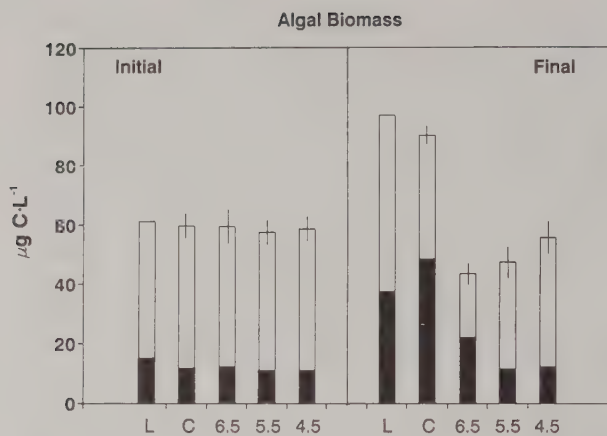


FIG. 2. Initial and final algal biomass in the lake and treatments. The black portion of each bar represents $<20\ \mu\text{m}$ algae. Vertical bars are ± 1 standard error.

Statistical Analyses

To determine whether observed intertreatment differences for the response variables of biomass, ^{14}C assimilation, and transfer efficiency were statistically significant, one-way ANOVAs were performed on the log-transformed initial and final data. Tukey's test was used as the mean separation technique. The term "significant" is used herein to indicate $\alpha < 0.05$. Analyses were done using SAS Stat version 5 (SAS Institute 1985) and the Kent State University IBM computer. For the initial data, $n = 3$ for all treatments. For the final data, $n = 3$ for the control and pH 6.5 treatments, and $n = 2$ for the pH 5.5 and 4.5 treatments; three bags developed holes prior to the final sampling.

Results and Discussion

The results permit testing of several hypotheses regarding plankton responses to lake acidification: (1) with declining pH, there is an increase in the relative biomass of large unproductive algal cells (Eriksson et al. 1980; Schindler 1990), (2) with declining pH, there is a reduction in the ratio of zooplankton/algal biomass (Olofsson et al. 1988; Yan and Welbourn 1990), (3) with declining pH, there is a reduction in the efficiency of energy transfer from producers to herbivores (Yan and Strus 1980), and (4) the reduced ecological transfer efficiency occurs because small zooplankton and large (inedible) algae simultaneously become dominant at low pH. The present results provide support for the first three hypotheses; the fourth requires further investigation.

Phytoplankton Composition

Algal biomass (Fig. 2) was $58\text{--}60\ \mu\text{g C}\cdot\text{L}^{-1}$ in all treatments and the lake at the start of the experiment. Roughly 75% of the biomass was due to "inedible" $>20\ \mu\text{m}$ algae (especially *Anabaena* sp. and *D. ehrenbergianum*). The "edible" fraction was dominated by nanoflagellates, *R. minuta*, *C. erosa*, and *Chlorella vulgaris*. In the final samples, total algal biomass in the lake and control had increased greatly, to 97 and $90\ \mu\text{g}\cdot\text{L}^{-1}$, and edible algae accounted for 40 and 56% of the biomass. The increases were largely due to $<5\ \mu\text{m}$ nanoflagellates. In the acidified treatments, biomass did not increase during the experiment; final algal biomass in the pH

6.5, 5.5, and 4.5 treatments was significantly lower than in the control. In the pH 6.5 treatment, 52% of the biomass was due to edible algae, especially nanoflagellates. At pH 5.5 and 4.5, only 22% of the biomass was edible, reflecting the lower biomass of nanoflagellates and the high relative biomass of the large cells *C. klebsii* ($24\ \mu\text{m}$) and *P. inconspicuam* ($25\ \mu\text{m}$). *Peridinium inconspicuam* was especially important in the pH 4.5 treatment, where it accounted for 60% of the final algal biomass. These changes in algal composition with declining pH are consistent with the findings of previous studies. Havens and DeCosta (1987) acidified water inside large mesocosms in soft-water Lake O'Woods, West Virginia. Mesocosms at pH 4.7 became dominated by *P. inconspicuam* (92% of biomass), while pH 7.0 controls were diatom and chlorophyte dominated. Similarly, when Havens and Heath (1990) acidified mesocosms in East Twin Lake, *P. inconspicuam* accounted for 27–64% of total biomass in a pH 4.5, high-Al treatment, while cyanobacteria, chrysophytes, and diatoms dominated in pH > 8 , low-Al controls. Overall, Havens and Heath (1990, 1991) reported a significant increase in mean algal cell size with acidification. During the 8-yr experimental acidification of Lake 223 (Experimental Lakes Area), dinoflagellates also increased in relative biomass (Findlay and Kasian 1986), and Schindler (1990) noted that mean algal cell size had increased. Multilake surveys have also shown that large dinoflagellates have greater relative biomass in lakes of lower pH (Yan 1979; Stokes and Yung 1986).

The lack of significant differences in total algal biomass among the pH 6.5, 5.5, and 4.5 treatments was also consistent with the results of previous research. It has often been demonstrated that acidification per se does not reduce algal biomass (Findlay and Kasian 1986; Havens and DeCosta 1985, 1987), and that the low algal biomass typical of acid lakes is due to the scarcity of limiting nutrients, especially P (DeCosta and Preston 1980; Wilcox and DeCosta 1982; Singer et al. 1984). In the present experiment, the mesocosms were sealed, frequently stirred for pH adjustment, and experienced little periphyton growth. Hence, there was likely no decline in total P due to the acidification. Algal biomass did increase in the lake and controls during the experiment, largely the result of nanoflagellates. These small cells occurred at a greatly reduced abundance in the low-pH treatments. It may be that the species comprising this group were acid sensitive, and experienced greater death rates at low pH.

Zooplankton Composition

Zooplankton biomass (Fig. 3) ranged from 87 to $103\ \mu\text{g C}\cdot\text{L}^{-1}$ in the initial samples; there were no significant differences among the treatments. Roughly 60% of the biomass was due to macrocrustacean herbivores, especially *S. oregonensis*, *D. retrocurva*, *D. birgii*, and *E. coregoni*. *Mesocyclops edax*, cyclopoid copepodids, nauplii, and rotifers (*K. cochlearis* and *Polyarthra vulgaris*) accounted for the remainder of the biomass. These same zooplankton species are common to soft-water lakes of northeastern North America (Keller and Pitblado 1984; Carter et al. 1986; Tessier and Horwitz 1988; Keller et al. 1990; Pinel-Alloul et al. 1990). In the final samples, zooplankton biomass had increased to 129 and $116\ \mu\text{g}\cdot\text{L}^{-1}$ in the lake and control. This was largely due to increases of the cladocerans *D. galeata mendotae*, *D. retrocurva*, *D. birgii*, and *E. coregoni*, which accounted for 50% of the total biomass. Their increases were likely due to the proliferation of edible nanoflagellates in the lake and control. Previous studies have shown rapid increases in cladoceran biomass following fertilization-induced enhancement of edible algal biomass in both

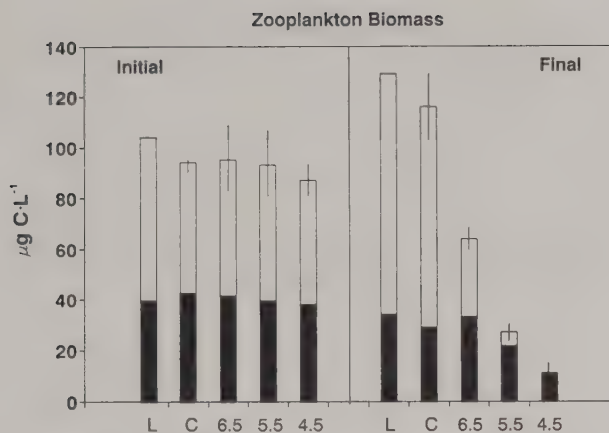


FIG. 3. Initial and final zooplankton biomass in the lake and treatments. The black portion of each bar represents microzooplankton herbivores (rotifers and nauplii) and carnivorous forms. Vertical bars are ± 1 standard error.

acid (DeCosta et al. 1983) and alkaline (Vanni 1987) lakes. Final zooplankton biomass was much lower in the acidified treatments, and showed a significant decline across the gradient of decreasing pH. At pH 6.5, macrocrustacean herbivores accounted for 48% of the total biomass, while they accounted for only 21 and 5% of the biomass at pH 5.5 and 4.5. At pH 5.5, the daphnids became extinct, and the biomasses of *E. coregoni*, *D. birgii*, and *S. oregonensis* were greatly reduced. Only *M. edax* (adults and immatures) and rotifers (*K. cochlearis* and *P. vulgaris*) were unaffected by acidification to pH 5.5. At pH 4.5, all adult crustaceans became extinct, and the zooplankton consisted of a low biomass of cyclopoid nauplii, copepodids, and rotifers. These acidification-induced changes are similar to those reported previously. Keller et al. (1990) found that *D. galeata mendotae* declined in lakes of pH < 6.0 , and that it was "absent from virtually all lakes with pH < 5.5 ." Carter et al. (1986) noted that *D. retrocurva* was excluded from acid waters in Atlantic Canada, and Pinel-Alloul et al. (1990) found that *D. galeata mendotae* biomass was positively correlated with alkalinity in Quebec lakes. In a survey of acid-impacted lakes in the north-eastern United States, Tessier and Horwitz (1988) found that *D. galeata mendotae* and *D. retrocurva* were absent in lakes of pH < 6.5 and 6.0, respectively. During the acidification of Lake 223, *D. galeata mendotae* became extinct when pH declined below 5.5 (Malley and Chang 1986). While large herbivores decline during acidification, rotifer biomass increases (Yan and Geiling 1985; Barmuta et al. 1990). This may be a response to the decline of large daphnids, which can hinder rotifers through competition (Gilbert 1988; MacIsaac and Gilbert 1991). Because of the short experimental duration, a trend of increasing rotifer biomass was not observed herein.

Zooplankton/Algal Biomass Ratios

The ratio of zooplankton/algal biomass (B_z/B_a) was roughly 1.6 in the initial samples, i.e. this portion of the pelagic biomass pyramid was inverted (Fig. 4). In the final samples, the ratio had changed little (1.4–1.5) in the lake, control, and pH 6.5 treatments. In the pH 5.5 and 4.5 treatments, the final ratios were significantly lower, at 0.7 and 0.2, respectively. These declines reflected the slight increase in algal biomass and the

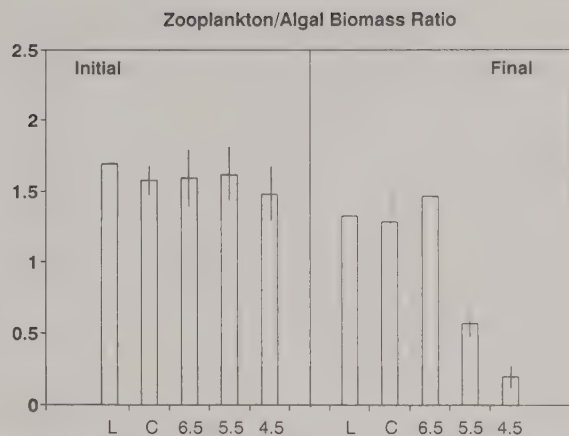


FIG. 4. Initial and final ratios of zooplankton/algal biomass in the lake and treatments. Vertical bars are ± 1 standard error.

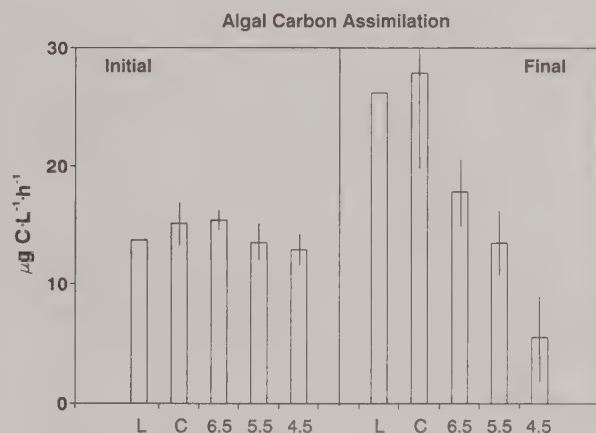


FIG. 5. Initial and final algal C assimilation rates in the lake and treatments. Vertical bars are ± 1 standard error.

great decrease in zooplankton biomass over the pH 6.5–4.5 gradient of treatments. Only a few studies have quantified the effects of acidification on this biomass ratio. The present results are supportive of the hypothesis that the ratio is reduced at low pH. Havens and DeCosta (1986) presented data indicating a great decline in the B_z/B_a ratio after they acidified lake water in a neutral-pH West Virginia lake. As in the present study, algal biomass increased and dinoflagellates became dominant, while zooplankton biomass declined. Olofsson et al. (1988), in summarizing results from Swedish lakes, noted that acidified lakes below pH 5 had B_z/B_a ratios 10–100 times lower than did higher pH lakes having less "unfavorable" algae. Yan and Welbourn (1990) reported a B_z/B_a ratio of 0.1 for acidic Clearwater Lake, and 0.3 for the less impacted Muskoka–Haliburton, Ontario, lakes. In the present experiment, the reduced B_z/B_a ratio was largely due to the loss of macrozooplankton, which as a group were more sensitive to the acidification than were the phytoplankton.

Algal C Assimilation

The initial rates of algal C assimilation (Fig. 5) were 13–16 $\mu\text{g C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$; there were no significant differences among the treatments. The final rates were markedly higher in both

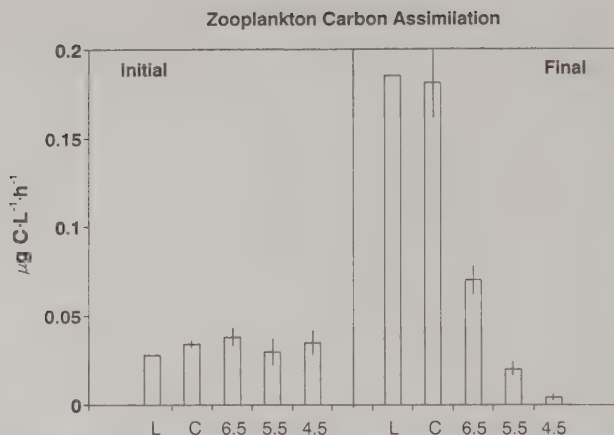


FIG. 6. Initial and final zooplankton C assimilation rates in the lake and treatments. Vertical bars are ± 1 standard error.

the lake and control, at 26 and 28 $\mu\text{g C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$, consistent with the increased algal biomass. Algal C assimilation rates declined in each successive treatment across the pH gradient from control to 4.5. At pH 4.5, the rate of algal C assimilation was significantly lower than in the control and pH 6.5 treatments. Because algal biomass increased slightly from pH 6.5 to 4.5, the decrease in C assimilation rates was likely due to the shift from small active nanoflagellates at pH 6.5 to large, less productive net plankton at pH 5.5 and 4.5. Indeed, P/B ratios calculated from the treatment C assimilation rates and algal biomasses were 0.39 (pH 6.5), 0.30 (pH 5.5), and 0.11 (pH 4.5). These results are supportive of the hypothesis of Eriksson et al. (1980) and Schindler (1990).

Zooplankton C Assimilation

Zooplankton C assimilation rates (Fig. 6) were below $0.05 \mu\text{g C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ in the lake and treatments at the beginning of the experiment; there were no significant intertreatment differences in the rates. These low rates of C intake likely reflected the dominance of filamentous cyanobacteria (especially *Anabaena* spp.) in the phytoplankton, which may have been an unsuitable food source for the herbivores. In contrast, final zooplankton C assimilation rates were nearly $0.2 \mu\text{g C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ in the lake and control, where the biomass of nanoflagellates had greatly increased. Previous research has shown that small naked cells are greatly exploited (Porter 1973) and readily assimilated (Schindler 1971) by zooplankton herbivores. It is the increase of such cells which likely led to the enhanced *Daphnia* densities in the lake and control and, in turn, to the increased zooplankton C assimilation rates.

Zooplankton C assimilation rates declined significantly across the gradient of control to 4.5 pH treatments, as the biomass of macrozooplankton was reduced. It is noteworthy that in another study of acidification and zooplankton grazing, no significant pH effects on community grazing rates were found (Sierszen and Frost 1990). However, in that study, comparing the control and acidified basins of Little Rock Lake, Wisconsin, pH differed by only 1 unit (6.2, 5.2). Furthermore, the same herbivore species were dominant in both basins, and *Daphnia* spp. was more abundant at pH 5.2. Hence, the Little Rock Lake zooplankton had not yet experienced the extinction of large herbivores that is typical of precipitation-acidified lakes.

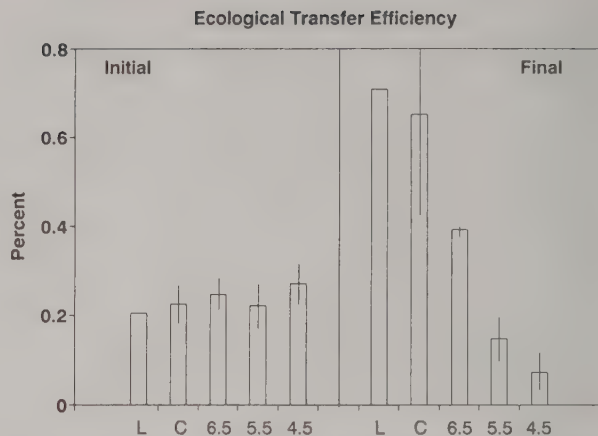


FIG. 7. Initial and final ecological transfer efficiencies (the ratio of zooplankton/algal C assimilation rate) in the lake and treatments. Vertical bars are ± 1 standard error.

Ecological Transfer Efficiency

Changes in the composition and activity of the algal and zooplankton communities were reflected in the ecological transfer efficiencies (Fig. 7). Initial values were low in all treatments and the lake, due to low rates of zooplankton C assimilation from the cyanobacteria-dominated phytoplankton. Greatly elevated rates were seen in the final lake and control samples, reflecting a more effective energy transfer when small edible nanoflagellates and large daphnids were abundant. The declining transfer efficiencies along the pH gradient were consistent with the hypothesis of Yan and Strus (1980), and reflected the great decline in macrozooplankton biomass.

The overall impacts of the treatments on the algal-zooplankton interface are illustrated in Fig. 8. In the lake and control, both algal and zooplankton C pools were large, and roughly 1% of the C assimilated by algae was subsequently assimilated by zooplankton. The relative biomass of edible algae and macrocrustacean herbivores was high. A similar situation existed at pH 6.5, except that the magnitude of all pools and C fluxes was lower. In the pH 5.5 and especially the pH 4.5 treatments, a great decline occurred in the size of the zooplankton C pool relative to the algal pool, and a lesser amount of the C assimilated by algae was transferred to zooplankton. These results were largely due to reduced zooplankton biomass, rather than to a lack of food for the herbivores where large algae were dominant. When zooplankton P/B ratios were calculated, the highest ratio (0.004) was found at pH 4.5, the lowest ratios at pH 6.5 and 5.5 (0.001), and intermediate ratios at pH 8.5 and in the lake (0.002), i.e. there was no consistent pattern of decline in zooplankton P/B ratio, as would be expected if zooplankton become less effective in acquiring algal C along a pH gradient. Yet, it is clear from previous research that the dominant large algae at pH 5.5 and 4.5 were unavailable to the microzooplankton herbivores (Havens and DeCosta 1985). Perhaps C flow through alternate pathways of the "microbial loop" (Azam et al. 1983) becomes increasingly important at low pH. This important question should be addressed in future research.

In conclusion, the results showed that by altering the composition and biomass of the plankton, acidification indirectly affected the functioning of the pelagic food web. Specifically, with declining pH, there was a reduction in ecological transfer efficiency in the grazing food chain, as hypothesized by Yan

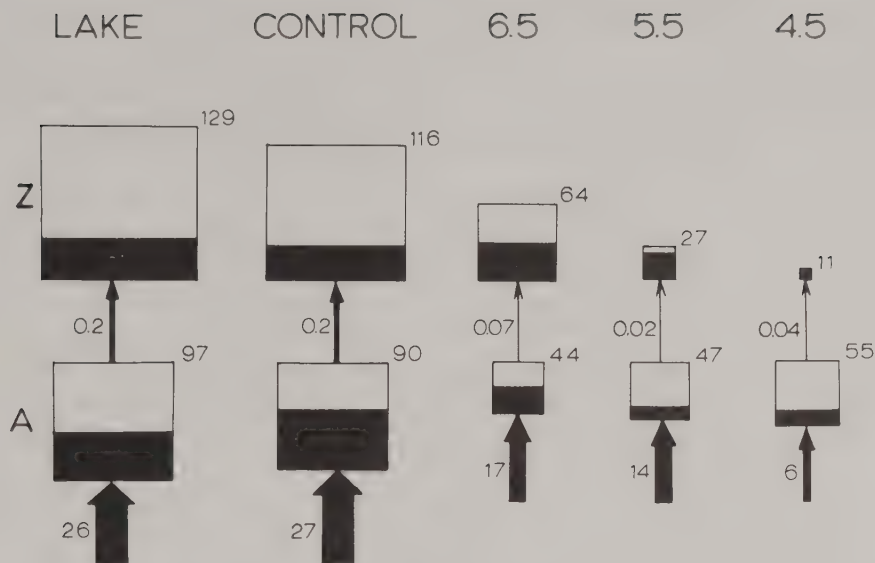


FIG. 8. Biotic C pools and pathways in the lake and treatments on days 9–10. The relative size of boxes and the numbers in the upper right corners indicate biomass ($\mu\text{g C}\cdot\text{L}^{-1}$). The thickness of arrows and the associated numbers indicate C assimilation rates ($\mu\text{g C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$). Upper boxes and arrows represent zooplankton (Z); lower boxes and arrows represent phytoplankton (P). In the phytoplankton boxes, the black portion represents $<20 \mu\text{m}$ cells. In the zooplankton boxes, the black portion represents microzooplankton herbivores and carnivores.

and Strus (1980). However, rather than being caused by the shift towards larger algae and smaller zooplankton dominance, the reduced efficiency was the result of a decline in zooplankton biomass. Further research is needed to test the hypothesis that a bottleneck to energy flow at the algal–zooplankton interface occurs in acidified lakes.

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Indices of Relative Abundance from Fish Spotter Data based on Delta-Lognormal Models

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Fish spotters are pilots in small aircraft employed by commercial fishermen to locate, identify, estimate the size of, and direct boats toward pelagic fish schools. Data describing species, location, and size of schools can be inexpensively obtained from fish spotters but are difficult to interpret statistically. We developed an index of relative abundance from fish spotter data based on extended delta-lognormal models and applied the method to data for northern anchovy (*Engraulis mordax*). In contrast with previous approaches, our method used all available data, provided an index for northern anchovy that was proportional to abundance, and explicitly modeled factors (pilots, regions, seasons, and time of day) that affected observations by fish spotters. We also included information about mixed layer depth and sea surface temperature in models for a reduced study area and found that environmental data, where available, can be used to improve estimates of relative abundance from fish spotter data. Simulation results indicated that our approach is a cost-effective way to improve biomass estimates for pelagic species like northern anchovy.

Les observateurs de poissons sont des pilotes de petits avions employés par des pêcheurs commerciaux pour localiser, identifier, évaluer la taille et diriger les bateaux vers les bancs de poissons pélagiques. Des données indiquant l'espèce, l'emplacement et la taille des bancs peuvent être obtenues à peu de frais des observateurs de poissons, mais elles sont difficiles à interpréter statistiquement. Nous avons élaboré un indice d'abondance relative à partir des données des observateurs de poissons basé sur des modèles delta-lognormaux étendus et nous avons appliqué la méthode à des données sur l'anchois du Nord (*Engraulis mordax*). Contrairement à celles utilisées précédemment, notre méthode utilise toutes les données disponibles, fournit un indice pour l'anchois du Nord qui est proportionnel à l'abondance et modélise explicitement les facteurs (pilotes, régions, saisons et heure) qui influent sur les observations effectuées par les observateurs de poissons. Nous avons également inclus de l'information sur la température en surface et en profondeur des couches mélangées dans les modèles pour une aire d'étude réduite et nous avons découvert que les données environnementales, lorsque disponibles, peuvent être utilisées pour améliorer les estimations d'abondance relative faites à partir des données fournies par les observateurs de poissons. Les résultats des simulations ont indiqué que notre méthode constitue une façon économique d'améliorer les estimations de la biomasse d'espèces pélagiques comme l'anchois du Nord.

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Information about changes in abundance of fish stocks is an important part of information required for fisheries management. Catch-per-unit-effort (CPUE) data from fisheries have traditionally been used to measure changes in relative abundance, but accumulated evidence indicates that interpreting CPUE data from commercial fisheries is difficult, particularly for pelagic fish, because commercial CPUE data are affected by changes in fishing efficiency over time and tend to decline more slowly than abundance (Bannerot and Austin 1983; MacCall 1984). A number of alternative fishery-independent procedures for estimating relative abundance of fishes have been developed including scientifically designed acoustic surveys, trawl surveys, egg and larva surveys, aerial surveys, and tagging experiments (Ulltang 1977). These approaches may provide good results but are expensive. In contrast, aerial "fish spotters" are a potential source of large quantities of inexpensive information about relative abundance of pelagic fish that can be obtained from existing commercial operations. To date, fish spotter data have received relatively little attention from fishery managers.

Fish spotters are pilots in small aircraft employed by commercial fishermen to locate, identify, estimate the size of, and

direct boats towards pelagic fish schools (Squire 1961; Maughan and Marmelstein 1971). Fish spotters are used in the Australian southern bluefin tuna (*Thunnus maccoyii*) fishery; the southern California purse-seine fishery for northern anchovy (*Engraulis mordax*), Pacific (chub) mackerel (*Scomber japonicus*), jack mackerel (*Trachurus symmetricus*), Pacific bonito (*Sarda chiliensis*), bluefin tuna (*Thunnus thynnus*), and Pacific sardine (*Sardinops sagax*); the South African purse-seine fishery for anchovy (*Engraulis capensis*) and pilchard (*Sardinops ocellata*); the Japanese sardine (*Sardinops melanosticta*) fishery; the U.S. Atlantic menhaden (*Brevoortia tyrannus*) and swordfish (*Xiphias gladius*) fisheries, and other pelagic fisheries around the world (Squire 1961, 1972, 1983; Maughan and Marmelstein 1971; Agenbag 1980; Anonymous 1981; Williams 1981; Habib et al. 1982; Agenbag et al. 1984; Hara 1985).

It is important to emphasize the differences between data from fish spotters and data from aerial surveys conducted for scientific purposes. Methods are available for using data from scientifically designed aerial surveys, usually in connection with acoustic data, to estimate biomass or relative abundance of pelagic fishes (Cram and Hampton 1976; Rivas 1978; Hampton et al. 1979; Hara 1986; Scott et al. 1989; Hara 1990). Data

collected during such surveys are relatively easy to interpret but costly. In contrast, data collected opportunistically during the search for fishable schools by fish spotters are harder to interpret but inexpensive (Anonymous 1981).

Accuracy and precision of fish spotter data are reinforced because inaccurate species identification and poor estimates of school size inconvenience fishermen who employ fish spotters. Difficulties with interpretation of fish spotter data arise, however, because of factors that are unique to fish spotter data (imprecise nature of visual biomass estimates and differences among fish spotters) and other factors (patchy distribution of pelagic fish, lack of systematic sampling design, environmental variables, differences among regions, seasons, and time of day) that hinder interpretation of other types of data (e.g. commercial CPUE) as well. Flights are conducted only when environmental conditions are good enough for fishing, but environmental conditions still affect observations by fish spotters. Changes in fish spotters employed by a commercial fishery are another important problem because changes in abundance may be confounded by changes in fish spotters who collect the data. Despite problems in interpretation, fish spotter data can be useful and cost-effective tools for management purposes (Barnes et al. 1992), particularly, as in our analysis, when adjustments are made for differences among fish spotters, regions, and other important factors.

An important advantage of fish spotter data over commercial CPUE data is absence of saturation (increased abundance but no increase in CPUE due to limited hold capacity, trip limits for catch, market demand, or processing capacity) because fish spotters can record the size and location of all schools seen. This important characteristic makes it relatively easy to construct indices of abundance based on fish spotter data that are linear measures of fish biomass (i.e. change in proportion to biomass). Another advantage is that technical improvements to equipment used by fish spotters, such as aircraft and navigational gear, have probably not substantially increased their efficiency in locating fish. Fish spotters may purchase new aircraft and better navigational or radio equipment, but schools are still located visually while flying at reduced speeds and low altitudes. In contrast, changes in commercial CPUE due to changes in fish abundance are often confounded by technical improvements to fishing gear such as nets and acoustic equipment (Kimura 1981; Jacobson et al. 1987).

We developed delta-lognormal linear models (Shimizu 1988) for fish spotter data based on the delta distribution (Aitchison and Brown 1957; Pennington 1983) and lognormal linear models (Bradu and Mundlak 1970; Kerlinger and Pedhazur 1973; McCullagh and Nelder 1983). Our approach extends delta-lognormal models and breaks new ground in fisheries because we model components of the delta distribution as functions of factors and covariates in lognormal linear models (Lambert 1992) and provide approximate variances for estimates. Lognormal linear models are similar to analysis of variance models fit using linear regression and often used by fishery scientists and managers to derive a single index of fishing effort for a group of heterogeneous vessels or to derive a single index of relative abundance for a fish stock from two or more types of data (Gulland 1956; Robson 1966; Kimura 1981, 1988; MacCall and Prager 1988; Hilborn and Walters 1992). Although we used lognormal linear models for components of the delta distribution, other linear or nonlinear models based on other statistical distributions could be used instead.

The delta distribution is often used to estimate abundance of planktonic organisms whose spatial distribution is highly con-

tagious (Dennis and Patil 1988). Survey data for planktonic organisms typically have statistical distributions with a large fraction of "zeros" (samples in which no organisms are observed) and may be so distorted that conventional methods based on the sample mean yield inefficient estimates of abundance. The delta distribution avoids problems with contagion by treating zero and nonzero data separately; final estimates of abundance are obtained from the product of the proportion and mean for nonzero observations. The delta distribution is used in many disciplines to model processes that generate more zero observations than might be expected on the basis of distributional assumptions (Lambert 1992).

Our approach used two lognormal linear models: the first to estimate the proportion of flights in which fish were seen by fish spotters and the second to estimate density (tons per area) of fish from data for flights during which fish were seen. Analogous to the delta distribution, our index of relative abundance from fish spotter data was based on the product of these quantities (adjusted for the size of the survey area as described below).

We applied our models to data for northern anchovy off the coast of southern California. In addition, we applied an expanded version of the model to a subset of the fish spotter data and two environmental variables (sea surface temperature and mixed layer depth) to determine if environmental information could potentially be used to improve estimates. Other types of environmental data (e.g. sea state and wind speed) were not available but may have been useful. The amount of data available and models used in our analysis enabled us to obtain useful estimates from fish spotter data and to account for differences among fish spotters, regions, seasons, time of day, and other variables.

Previous analyses of fish spotter data used a simple CPUE-like index (tons sighted per block area flight or T/BAF) to measure relative abundance (Squire 1961, 1972, 1983). The T/BAF index was corrected for time of day by excluding data for day flights and corrected for differences among regions by restricting the analysis to a "core" region where the species of interest was naturally abundant. A significant advance in our analysis was that all available information was used to estimate abundance; corrections for differences between time of day, among regions, and seasons were made without excluding data.

Another important difference between our analysis and previous ones was that we made corrections for differences among fish spotters. Our records indicated consistent differences among fish spotters in mean reported weight of anchovy schools and the proportion of flights during which schools were sighted (Fig. 1). Moreover, fish spotters participating in the monitoring program changed over time so that, for example, some fish spotters participated only during the 1960's and 1970's, while others did not begin to participate until the late 1980's. If indices of abundance from fish spotter data were not corrected for differences among fish spotters, then temporal changes in abundance may have been confounded by changes in fish spotters. As shown below, effects of fish spotters were important for anchovy.

The original motivation for our work was to improve spawning biomass estimates used to manage the fishery for northern anchovy. Data for biomass estimates were reduced in recent years and precision and accuracy of biomass estimates deteriorated (Jacobson and Lo 1990). For this reason, we conducted simulation experiments to determine if our new index based on fish spotter data could be used to improve biomass estimates and management advice for northern anchovy.

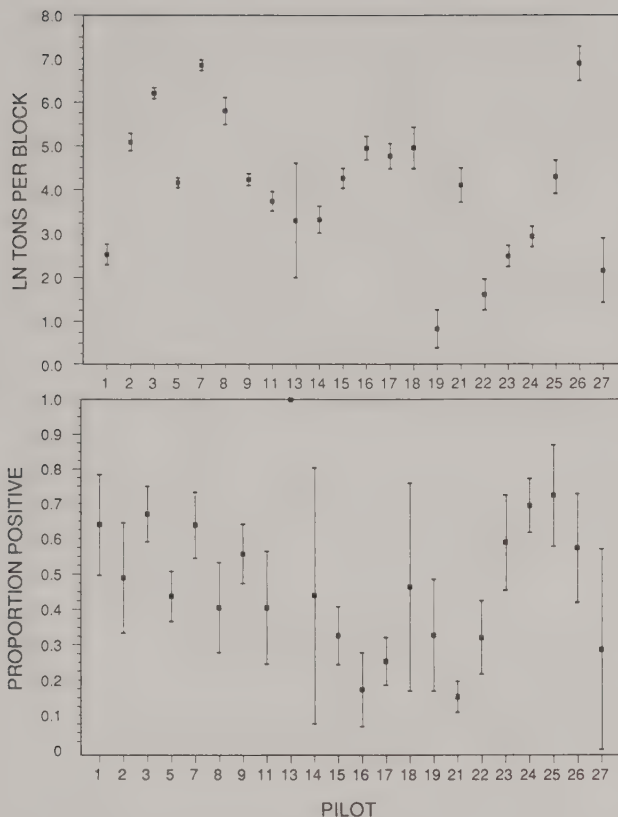


FIG. 1. Mean log tons of anchovy sighted per block (top panel) and proportion positive blocks (bottom panel) for fish spotters in region 2 (see Fig. 2) during 1963–90. Some fish spotters did not participate during the entire time period. Vertical bars show means $\pm$ 2 SE.

Materials and Methods

The anchovy data used in our analyses were collected by fish spotters paid a nominal wage (\$1 to \$4 per hour) to participate in a pelagic resource monitoring program initiated in 1962 by the Tiburon Marine Laboratory, U.S. Fish and Wildlife Service (now Southwest Fisheries Science Center, National Marine Fisheries Service, or NMFS), in central and southern California (Squire 1961, 1972). Data were collected during the course of routine fish spotting and involved little additional effort by fish spotters. The data set (called the “complete” data set below) included records from about 16 000 flights conducted during 1963–90 (records for fish spotters with less than 20 flights were omitted). The subset of fish spotter data used with environmental data consisted of records from about 3700 flights over a smaller geographic area (records for fish spotters with less than 10 flights were omitted).

Fish spotters recorded the species (most pelagic schooling fishes of commercial importance can be identified from the air), location, time of day, and estimated weight of all fish schools encountered during flights in special flight logs (Squire 1961, 1972). Locations were specified by “blocks” which are 10' latitude by 10' longitude. Time of day was either day or night (fish schools are visible at night due to bioluminescence; Squire 1972). Flight log data for each sighting were checked for errors and entered into a computer database.

The area covered by fish spotters was divided into six regions (Fig. 2). During 1963–90, fish spotters collected data from the area between 27 and 37°N latitude, but anchovy were most common in the area north of region 6 (30°33'N latitude). Data for region 6 were excluded from our analysis because anchovy were seldom seen there.

Sea surface temperature and mixed layer depth were estimated for each block using data collected during routine California Cooperative Oceanic Fisheries Investigations (CalCOFI) research cruises (L. Eber, NMFS, Southwest Fisheries Science Center, P.O. Box 271, La Jolla, CA, 92038 USA, pers. comm.). CalCOFI cruises were conducted throughout the study period (with some interruptions) on a quarterly basis (Hewitt 1988). Environmental data could be obtained for regions 2 and 3 (Fig. 2) only because CalCOFI cruises have been limited to these regions since 1987. Years with no CalCOFI environmental data were omitted from our models when environmental data were included.

Sea surface temperature and mixed layer depth were treated as categorical, rather than continuous, variables because initial estimates of the functional form of the relationships between anchovy biomass and sea surface temperature or mixed layer depth were erratic when environmental data were treated as continuous variables. Although preliminary estimates of functional forms were uninterpretable, it was clear that anchovy abundance varied with sea surface temperature and mixed layer depth when the environmental data were treated as categorical variables. Sea surface temperature data were, therefore, aggregated in 2°C categories and mixed layer depth data were aggregated into 20-m categories. Intervals for the categories were chosen so that there was a sufficiently large number of observations in each category.

Models

As described above, relative abundance of northern anchovy was expressed as the product of density and a measure of area:

$$(1) \quad I = DA$$

where I is the index of relative abundance for a given year (tons), D is density of anchovy (tons per block), and A is the area (blocks) covered by fish spotters. We assumed that fish spotters flew over an area that was at least as large as the area occupied by the anchovy stock in each year. Units for the index (I) are tons of anchovy sighted by fish spotters.

Density of anchovy (D) for each year was

$$(2) \quad D = dP$$

where d is a standardized measure of anchovy density (tons per block) for positive flights (flights during which anchovy were seen) and P is a standardized measure of the proportion of blocks that were covered by positive flights (referred to as proportion positive). As described above, density of anchovy (D) was calculated from the product of density in positive blocks (d) and proportion positive (P) in order to avoid problems that arise from including a large number of zeros. Moreover, area of the stock (A), density for positive flights (d), and proportion positive (P) are all useful measures of relative abundance for pelagic species (MacCall 1990; Mangel and Smith 1990; Smith 1990), while area (A) and proportion positive (P) provide information about spatial distribution.

The complete data set was used to obtain standardized estimates of anchovy density for positive blocks (d) and proportion positive (P) for each year using lognormal linear models

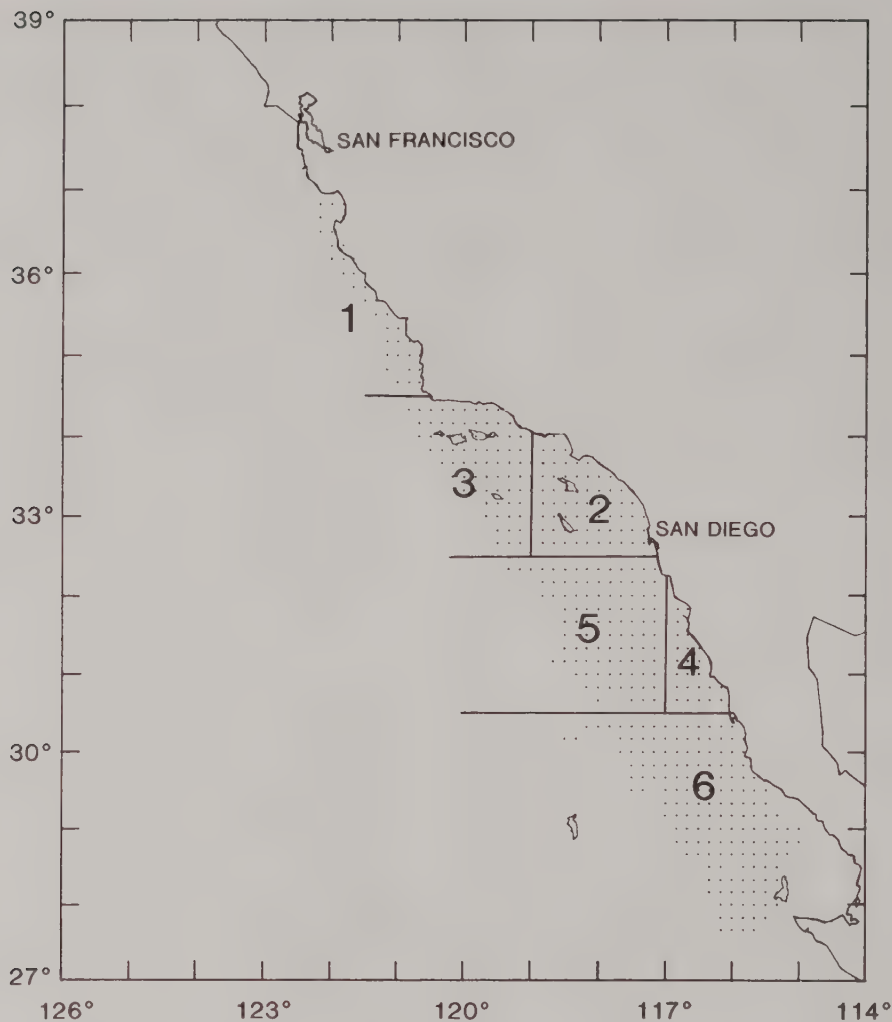


FIG. 2. Study area, regions, and blocks covered by fish spotters in 1989. Regions are outlined and denoted by numbers. Blocks are denoted by dots.

described below. Five factors were treated as main effects: years, fish spotters, regions, seasons, and time of day (day or night). Factors were allowed to cross in the model so that any combination of years, fish spotters, regions, seasons, and time of day could be accommodated. In addition to main effects, first-order interactions between regions, seasons, and time of day were included in the models. Other first-order and higher order interactions were not included to avoid overparameterization of models and problems with parameter estimation as well as to limit the amount of computer memory required.

Data used to estimate density of anchovy for positive flights (d) were aggregated by flight. The model was

$$\ln(d) = X\beta + Z\alpha + \epsilon$$

where d is a vector for observations, X is the design matrix for main effects and β is the parameter vector for main effects, Z is the design matrix for interactions and α is the parameter vector for interactions, and ϵ is a vector of independent normally distributed errors with expectation zero and variance σ^2 .

For record k , we have

$$(3) \quad \ln(d_k) = \ln(y_k/b_k) = f(\beta, \alpha, X_k) + \epsilon_k$$

and

$$(4) \quad f(\beta, \alpha, X_k) = \beta_0 + \sum_{i=1}^5 \sum_{w=1}^{J_i} \beta_{iw} X_{iwk} + \sum_{i=1}^5 \sum_{w=1}^{J_i} \sum_{L>1}^5 \sum_{v=1}^{J_L} \alpha_{iwlv} X_{iwk} X_{Lvk}$$

where d_k is tons of anchovy sighted per block for positive flight k , b_k is the total number of blocks searched during positive flight k (not the number of positive blocks), y_k is total tons of anchovy sighted, and ϵ_k is a normally distributed error with expectation zero and variance σ^2 . The number of positive blocks (b_k) was incremented each time a fish spotter left one block and entered another so that an individual block searched twice during a flight would be counted twice.

Subscripts i and L in (4) index main effects (years, fish spotters, regions, seasons, and time of day), w and v index factor levels (e.g. spring, summer, and autumn for seasonal effects), J_i is the number of levels for factor i , J_L is the number of levels for factor L , and k is used to index flights or records. The coefficient β_0 is the mean log density ($\ln(d_k)$) of anchovy sighted

TABLE 1. Symbols and descriptions for main effect and interaction parameters in lognormal linear models. The symbols “ w ” and “ v ” are used to subscript factor levels. The symbols “ α ” and “ β ” are used in models for density, while “ γ ” and “ τ ” are used in models for proportion positive.

Parameter	Description
α or γ	Vector for interaction parameters
β or τ	Vector for main effect parameters
β_0 or τ_0	Mean log density ($\ln(d_k)$) or mean log proportion positive ($\ln(P_k + 1)$) of anchovy sighted for a reference year (1963), region (region 2), season (winter, January–March), time of day (nighttime), fish spotter (fish spotter 1), and, if applicable, set of environmental conditions
β_{1w} or τ_{1w}	Year effects; $w = 1$ –27 for years 1964–90
β_{2w} or τ_{2w}	Region effects; $w = 1$ –4 for regions 1, 3, 4, and 5
β_{3w} or τ_{3w}	Seasonal effects; $w = 1$ –3 for spring (April–June), summer (July–September), and autumn (October–December)
β_{4w} or τ_{4w}	Day/night effect; $w = 1$ for daytime
β_{5w} or τ_{5w}	Fish spotter effects; $w = 1$ –21 for fish spotters numbered 2–27 (some fish spotters with less than 20 flights excluded)
β_{6w} or τ_{6w}	Temperature effects; $w = 1$ –4 for temperatures $\leq 12^\circ\text{C}$, > 12 and $\leq 14^\circ\text{C}$, > 16 and $\leq 18^\circ\text{C}$, and $> 18^\circ\text{C}$
β_{7w} or τ_{7w}	Mixed layer depth effects; $w = 1$ –3 for mixed layer depths ≤ 20 m, > 20 and ≤ 40 m, and > 60 m
α_{2w3v} or τ_{2w3v}	Interaction between regions and seasons; $w = 1$ –4 and $v = 1$ –3
α_{3w4v} or τ_{3w4v}	Interaction between seasons and time of a day; $w = 1$ –3 and $v = 1$
α_{2w4v} or τ_{2w4v}	Interaction between regions and time of a day; $w = 1$ –4 and $v = 1$

for a reference year (1963), region (region 2), season (winter, January–March), time of day (nighttime), and fish spotter (fish spotter 1). The vectors β and α hold parameters for main effects and interactions (see Table 1 for details). The vector X_k holds the dummy variables X_{iwb} (Weisberg 1980) for main effect i , level w , and positive flight k . The number of parameters and dummy variables for each main effect was equal to the number of categories minus 1. There were, for example, three parameters for seasonal effects with three dummy variables that were 0,0,0 for winter (January–March) flights 1,0,0 for spring (April–June) flights, 0,1,0 for summer (July–September) flights, and 0,0,1 for autumn (October–December) flights.

We used a stepwise linear regression program (BMDP2R; Dixon et al. 1988) to obtain maximum likelihood estimates for parameters in (3) and (4). The stepwise regression program sequentially applied forward selection and backward elimination algorithms to avoid problems associated with using one or the other exclusively (Weisberg 1980; Draper and Smith 1981), and statistical significance was established if the F -value for parameters associated with a main effect or interaction exceeded 4.0. The stepwise regression program made it easy for us to identify and select groups of parameters for main effects and

interactions in the model that were statistically significant (i.e. explained a significant amount of variance in the dependent variable; see Kerlinger and Pedhazur 1973). In a few cases, we forced the program to include an insignificant main effect or effects associated with a significant interaction so that, for example, both main effects A and B would be included if the interaction between A and B was significant. Changes in the coefficient of determination (R^2) and order in which the stepwise regression procedure included variables and interactions were used as crude measures of their relative importance.

A standardized and unbiased measure of anchovy density in positive flights during year j is $d_j = \exp(\beta_0 + \beta_{1j} + \sigma^2/2)$ where β_0 is mean log density ($\ln(d_k)$) for the reference year, region, season, time of day, and fish spotter and β_{1j} is the main effect for year j . This measure was estimated by

$$(5) \hat{d}_j = \exp(\hat{\beta}_0 + \hat{\beta}_{1j}) \Psi_{d_j}$$

where “hats” (^) denote estimates from fitting the lognormal linear model (3) to data and Ψ_d is a correction for bias. The correction for bias and variance of d_j were calculated as described in Appendix 1.

Data used to estimate proportion positive (P) were aggregated by year, fish spotter, region, time of day, and season in order to reduce the size of the data set. Preliminary analyses indicated that effects of different approaches to aggregating data were minimal. A lognormal linear model similar to (3) and (4) was used to estimate proportion positive:

$$(6) \ln(P_k + 1) = \ln(f_k/B_k + 1) = f(\tau, \gamma, X_k) + \zeta_k$$

where P_k is proportion positive for record k . Positive blocks (f_k) is the total number of blocks searched during positive flights and B_k is the total number of blocks searched during all flights that contribute to record k . The additive constant 1 in (6) allowed logarithmic transformation when P_k was zero. Choice of additive constant can effect results from analysis of variance models (Berry 1987). The additive constant 1 was a reasonable choice for our data because it usually reduced and never increased skewness after the transformation, while smaller values (e.g. 0.1) sometimes increased skewness. The linear function $f(\cdot)$ in (6) is the same as in (3) and (4) except that the vectors τ and γ hold parameters for main effects and interactions (Table 1). The term ζ_k was assumed to be a normally distributed error with expectation zero and variance δ^2 .

The assumption of normally distributed errors (ϵ_k and ζ_k) in (3) and (6) was reasonable because of the Central Limit Theorem (values of P_k were averages usually computed from a large number of observations) and because scatter diagrams of residuals were symmetric. A natural alternative would have been to assume binomial-distributed errors and estimate proportion positive (P_k) by logistic regression or other likelihood-based procedures (McCullagh and Nelder 1983). We chose to assume normally distributed errors because software for lognormal linear models facilitated computation of approximate variances for proportion positive and our index of relative abundance (Appendix 1). The assumption of constant variances in (3) and (6) was reasonable because scatter diagrams of residuals did not show any trend when plotted against predicated values or independent variables.

A standardized and unbiased measure of proportion positive during year j is $P_j = \exp(\tau_0 + \tau_{1j} + \delta^2/2) - 1$ where τ_0 is the mean of log proportion positive ($\ln(P_k + 1)$) for a reference year, region, season, time of day, and fish spotter and τ_{1j} is the main effect for year j . This measure was estimated by

$$(7) \hat{P}_j = \exp(\hat{\tau}_0 + \hat{\tau}_{1j}) \Psi_{P_j} - 1$$

where Ψ_p is a correction for bias and estimates were obtained by fitting the lognormal linear model (6) to data. The correction for bias and variance of $\hat{P}_j$ were calculated as described in Appendix 1.

Substituting (2) into (1) and replacing parameters with their estimates from (5) and (7) gives the following estimate of relative abundance for anchovy in year j :

$$(8) \quad \hat{I}_j = \hat{d}_j \hat{P}_j A_j \\ = [\exp(\hat{\beta}_0 + \hat{\beta}_{1j}) \Psi_{\hat{d}_j}] [\exp(\hat{\alpha}_0 + \hat{\alpha}_{1j}) \Psi_{\hat{P}_j} - 1] A_j.$$

Variance of (8) was estimated as described in Appendix 1. Models and procedures used with environmental and fish spotter data were similar to those used with the complete data set.

The structure of models (3) and (6) allowed us to estimate abundance of anchovy in the last year of the time series from data for only one season in that year. The estimates obtained in our analysis for 1990, for example, were based on data for January–March, while estimates for all other years were based on data for January–December. Use of data for one season or partial season in the last year decreases precision but makes it possible for managers to make estimates, which may be important in some circumstances, of relative abundance during the most recent or current fishing season. Another approach is to use annual periods other than calendar years so that the last season with data is the end of the last annual period (Jacobson and Lo 1991).

Results and Discussion

For the complete data set, the stepwise regression procedure included the same variables and interaction terms in the model (3) for density (d) and as in the model (6) for proportion positive (P) (Table 2). Effects due to time of day, regions, seasons, and fish spotters were all more pronounced than year effects (which are used in (8) to estimate relative abundance) in both models (Table 2). Final estimates for all indices (corrected for bias) are given with standard errors in Table 3.

Results for the environmental and subset of the fish spotter data were similar to those for the complete data set except that

the stepwise regression procedure included sea surface temperature in the model (3) for density (d) and both temperature and mixed layer depth in the model (6) for proportion positive (P). Although effects due to sea surface temperature and mixed layer depth were less pronounced than year effects in both models, these results indicate that environmental data were useful in interpreting fish spotter data. Final estimates for all indices (corrected for bias) are given with standard errors in Table 4.

Additional information about the importance of environmental variables can be obtained by examining results for 1983 which was an El Niño year with unusually warm water temperatures in the study area (Fiedler et al. 1986). The estimate of relative abundance (I) for anchovy during 1983 from the complete data set was implausibly low relative to the estimates for 1981, 1982, and 1984 due to low density (d) and proportion positive (P) (Fig. 3; Table 3), while the coefficient of variation (CV) for 1983 was higher than for adjacent years (Table 3). In contrast, the estimate of relative abundance (I) during 1983 and the coefficient of variation from fish spotter and environmental data (Fig. 3; Table 4) were more similar to estimates for 1981 and 1984 (no estimate for 1982 was available because of no environmental data). This result indicates that environmental data may be important in interpreting fish spotter data, particularly, as in El Niño years, when unusual environmental conditions exist.

Total area covered by fish spotters in each year (A) increased from 180 blocks in 1963 to around 350 blocks in the late 1980's (Table 3; Fig. 4). The number of blocks in which anchovy were sighted averaged about 100 blocks during 1963–84 and about 150 blocks during 1985–90. Since the number of blocks covered by fish spotters in each year was larger than the number of blocks in which anchovy were seen, we concluded that there was little bias in our estimates of relative abundance (I) due to fish spotters failing to cover the entire range of the anchovy stock.

Evaluation of Indices

We evaluated indices of relative abundance from fish spotter data by comparing them with estimates of total anchovy bio-

TABLE 2. Summary of lognormal linear models for density in positive blocks (d) and proportion positive (P) fit to the complete fish spotter data set for northern anchovy. Columns give the order of entry and change in R^2 for main effects and interactions included in the models. Numbers in parentheses indicate the number of parameters estimated for each variable or interaction. The row labeled "Records" gives the total number of flights (in the model for density) or records (in the model for proportion positive) used to estimate parameters.

	Density (d)		Proportion positive (P)	
	Order	Change in R^2	Order	Change in R^2
Intercept		Included in all models		
Time of day	1	0.088 (1)	3	0.0016 (1)
Regions	3	0.028 (4)	1	0.056 (4)
Fish spotters	2	0.26 (21)	4	0.105 (21)
Seasons	5	0.0127 (3)	2	0.025 (3)
Years	6	0.058 (27)	5	0.058 (27)
Region by time				
of day interaction	4	0.0217 (3) ^a	6	0.0172 (4)
Season by time				
of day interaction	8	0.0016 (3)	7	0.009 (3)
Region by season				
interaction	7	0.015 (10) ^a	8	0.011 (10) ^a
Records	6793		3733	
Total R^2 (adjusted)		0.48		0.24

^aSome coefficients were not estimated because of collinearity with other independent variables.

TABLE 3. Relative abundance of anchovy during 1963–90 and other estimates from the complete fish spotter data set. *A* is the area (number of unique blocks) covered by fish spotters in each year, number positive is the number of blocks in which anchovy were seen, *d* is density (tons per block) of anchovy for positive flights, *P* is proportion positive, *I* is relative abundance of anchovy, and CV is the coefficient of variation. Estimates of density (*d*) and proportion positive (*P*) were corrected for bias as described in the text.

Year	<i>A</i>	Number positive	<i>d</i> ^a	CV(<i>d</i>)(%)	<i>P</i>	CV(<i>P</i>)(%)	<i>I</i>	CV(<i>I</i>)(%)
1963	180	126	13	22	0.47	15	1 129	27
1964	206	118	76	26	0.61	16	9 569	30
1965	208	112	67	25	0.56	17	7 851	31
1966	224	106	109	24	0.60	16	14 643	29
1967	200	108	132	23	0.72	14	19 105	27
1968	221	106	50	25	0.48	19	5 260	31
1969	223	90	163	23	0.51	18	18 663	29
1970	143	75	167	24	0.71	16	16 965	29
1971	175	82	156	23	0.70	15	19 082	27
1972	184	87	154	23	0.67	16	19 025	28
1973	338	146	339	23	0.64	15	73 493	28
1974	304	96	255	23	0.76	14	58 892	27
1975	280	68	392	23	0.84	14	91 789	27
1976	388	97	221	23	0.63	16	53 966	28
1977	289	99	248	23	0.71	15	50 683	27
1978	283	84	358	25	0.52	18	52 898	31
1979	288	88	590	25	0.54	19	91 366	31
1980	196	100	455	29	0.69	19	61 345	34
1981	232	110	313	29	0.46	23	33 281	37
1982	249	88	264	28	0.42	24	27 846	37
1983	340	79	78	36	0.27	33	7 125	49
1984	341	82	176	32	0.29	31	17 373	45
1985	357	151	349	30	0.44	22	54 261	38
1986	332	122	132	29	0.42	23	18 289	38
1987	352	119	114	31	0.43	24	17 103	40
1988	335	201	275	31	0.50	22	46 164	38
1989	379	142	81	32	0.47	23	14 373	40
1990	351 ^b	147 ^b	227	42	0.41	33	32 527	54

^aRounded to nearest integer.

^bArea (*A*) and number positive for 1990 estimated from the average values for 1985–89.

mass during 1964–86 (Fig. 3) obtained independently by catch-at-age analysis using a stock synthesis model (Methot 1989). The stock synthesis model is a form of catch-at-age analysis that incorporates information about relative abundance of spawning or schooling anchovy from four fishery-independent surveys, commercial fishery data, and environmental data in a single maximum likelihood based procedure. Although the stock synthesis model for northern anchovy was designed to estimate spawning biomass, total biomass estimates are produced as well (Methot 1989; Lo and Methot 1989). Stock synthesis estimates of anchovy biomass have been used to set catch quotas since 1986 and were the best estimates available.

We also compared our estimates of relative abundance with updated versions of the simple T/BAF index from fish spotter data (Fig. 3) described by Squire (1972, 1983) to determine if our more complex approach was any better.

When comparing indices, it is important to remember that estimates of relative abundance from fish spotter data measure changes in *schooling* biomass rather than total, catchable, or spawning biomass. These distinctions may be important if, for example, young fish do not school or a large fraction of the stock is immature.

The first step in evaluating indices was to determine which were linear measures of relative biomass for northern anchovy. We fit a quadratic polynomial (parabolic) regression model to each index of relative abundance:

$$(9) \quad L_y = \iota_1 B_y + \iota_2 B_y^2 + \epsilon_y$$

where L_y is an index that measures biomass in year *y* (e.g. T/BAF), B_y is anchovy biomass during year *y* estimated by Methot (1989) using a stock synthesis model, ι_1 and ι_2 are parameters, and ϵ_y is a normally distributed statistical error. The important features of this model are that it intersects the origin (indices have zero value at zero anchovy biomass) and nonlinearity in the relationship between an index and anchovy biomass is indicated by the sign and statistical significance of the estimate of ι_2 . If the index of abundance (L) is not a linear estimate of anchovy biomass (B), then the estimate of ι_2 should be significantly different from zero. Negative values of ι_2 indicate saturation. The parabolic model (9) worked well for our data because the inflection point of the fitted line fell outside the range of the data for each index and the line was always increasing in the range from zero to the largest observed biomass level.

Results from the regression analyses (Table 5) indicate that our index of relative abundance from fish spotter data (*I*) and T/BAF were linear measures of relative anchovy biomass (Table 5). Parameter estimates for ι_2 were not statistically significant (*p*-value > 0.05) for our index of relative abundance from fish spotter data (*I*, with and without environmental data), T/BAF, and, in the case of fish spotter with environmental data, proportion positive (*P*).

To further evaluate linear measures of relative biomass for anchovy, we computed correlation coefficients between indices from fish spotter data and stock synthesis estimates of anchovy biomass:

TABLE 4. Relative abundance of anchovy during 1963–90 and other estimates from a subset of the complete fish spotter data plus environmental data. Column headings and definitions as in Table 3. Area (A in (8)) used to estimate relative abundance from fish spotter and environmental data was the same as listed in Table 3 for a complete data set. Estimates for some years are unavailable due to a lack of environmental data.

Year	d^a	CV(d)(%)	P	CV(P)(%)	I	CV(I)(%)
1963	41	33	0.19	9	1 366	34
1964	532	43	0.16	14	17 452	45
1965	368	45	0.16	15	12 076	47
1966	864	35	0.15	12	29 311	37
1967	820	40	0.17	13	28 634	42
1968	245	40	0.08	25	4 126	48
1969	943	33	0.13	13	27 583	36
1970						
1971						
1972	810	35	0.13	13	19 449	37
1973			No environmental data available			
1974	819	37	0.30	9	74 747	38
1975	2 564	34	0.25	8	179 773	35
1976	676	41	0.10	25	26 526	48
1977	645	40	0.11	21	20 911	46
1978	1 804	39	0.06	30	29 046	49
1979	1 153	77	0.06	48	19 729	91
1980	1 809	57	0.17	19	60 890	60
1981	1 057	52	0.11	22	26 877	56
1982						
1983	520	57	0.03	75	5 389	94
1984	562	57	0.04	57	8 387	81
1985	891	56	0.05	50	16 123	76
1986	794	57	0.05	52	12 687	77
1987	868	57	0.05	53	14 566	78
1988	434	63	0.02	129	3 166	144
1989	2 052	67	0.06	46	45 568	81
1990	1 445	63	0.07	48	33 333	79

^aRounded to nearest integer.

Index	Correlation with stock synthesis estimates
T/BAF	0.60
I (no environmental data)	0.67
I (with environmental data)	0.63

Indices of relative abundance (I) for anchovy based on delta-lognormal linear models appear superior to the T/BAF index because correlations with stock synthesis estimates of total biomass were slightly higher and because the T/BAF index dropped to implausibly low levels after 1982 (Fig. 3).

The correlation between stock synthesis estimates of biomass and our estimates of relative abundance (I) from environmental plus fish spotter data was almost as good as the correlation with estimates from the complete data set even though the sample size for each year was smaller. This result indicates that environmental data, if readily available, could be used to improve the precision of relative abundance indices from fish spotter data.

Simulation of Anchovy Biomass Estimates

Two simulation experiments involving the stock synthesis model for anchovy were conducted to determine if our index of schooling biomass (I) could be used to improve spawning stock biomass estimates for northern anchovy. The stock synthesis model was used in the simulation experiment because it was convenient and actually used to manage anchovy, but any

other type of catch-at-age analysis with auxiliary information (Deriso et al. 1985; Pope and Shepherd 1985; Gavaris 1988) would have been just as suitable. Our results are general enough to indicate the relative benefits of including fish spotter data in catch-at-age analysis with auxiliary information for fisheries similar to the anchovy fishery. A detailed description of the simulations is given in Appendix 2.

Simulation results indicated that fish spotter data increased the precision of biomass estimates from the stock synthesis model by about 10% on average (Fig. 5). Coefficients of variation for spawning biomass estimates with fish spotter data ranged from 7.8 to 40% in the last year and ranged from 7.6 to 54% in the last year without fish spotter data.

Fish spotter data may have reduced bias in stock synthesis estimates of spawning biomass for northern anchovy. Bias of stock synthesis estimates for each year was measured by the percentage difference between the mean estimate from the simulations and the "true" value assumed in the simulation experiment, i.e. as $(I_{\text{average}} - I_{\text{true}})/I_{\text{true}} \times 100$. Percentage difference is a reasonable measure of bias when the number of simulations employed is large but may be misleading when the number of simulations is as small as 50 because the mean of a small sample will differ from the underlying population mean due to sampling error only (Efron 1982).

The absolute value of our measure of bias ranged from 0 to 10% (0–63 000 tons) of true values in the simulation for relative abundance estimates from fish spotter data and from 0 to 12% (0–75 000 tons) for relative abundance estimates without fish spotter data (Fig. 5). As explained above, however, these

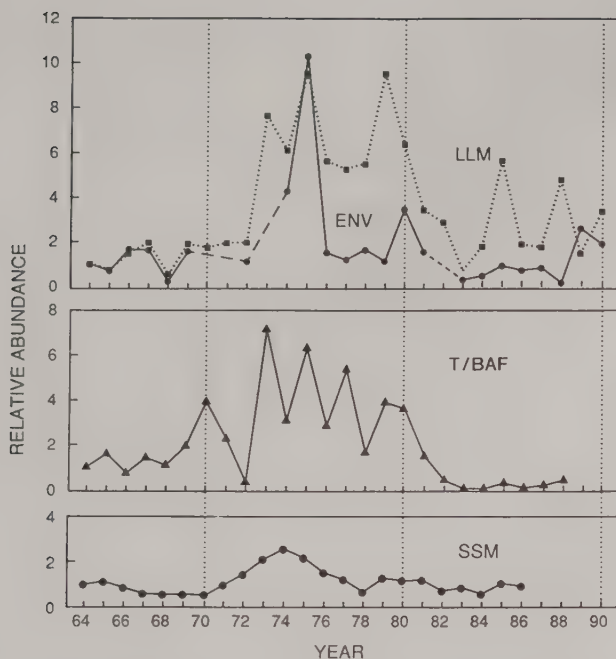


FIG. 3. Stock synthesis model (SSM) estimates of total biomass (Methot 1989), a catch-per-unit-effort-like index (T/BAF) from fish spotter data (revised and updated values similar to data given in Squire 1972), and estimates of relative abundance from delta-lognormal linear models fit to fish spotter data with (ENV) and without (LLM) environmental data. Before plotting, each series was scaled so that the value of 1964 was equal to 1.0 (i.e. the values in each series were divided by the value for 1964).

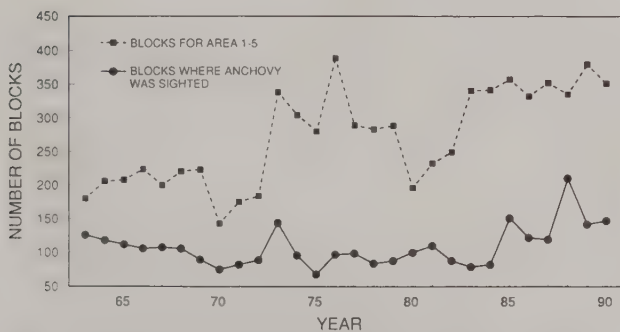


FIG. 4. Number of blocks in regions 1-5 covered by fish spotters (A) and number of blocks where anchovy were found during 1963-90.

results were based on relatively few simulations and may be misleading. Additional work is required to determine the nature and extent of possible bias in the stock synthesis model.

The most important effect of using fish spotter data in the simulations was increased precision and potential reduction in bias for the last year's estimate. The biomass estimate for the last year is the least precise in most stock assessments (e.g. Rivard 1989) but also the most important because it is used to set the next catch quota. Use of fish spotter data in our simulation experiment resulted in an improvement of 26% in the precision of spawning biomass estimates for the last year and may have reduced bias by 17% (i.e. $[12\% - 10\%]/12\%$). These results indicate that indices of relative abundance based

TABLE 5. Sign (+ or -) for ι_2 and its p -value under the null hypothesis that $\iota_2 = \text{zero}$ for regression analyses of anchovy abundance indices (equation (9)). Negative estimates indicate saturation. Small p -values (e.g. $p < 0.05$) indicate nonlinearity.

Index	Estimate for ι_2	
	Sign	p -value
T/BAF	-	0.910
Area (A)	-	0.000
Indices from fish spotter data only		
Density for positive blocks (d)	-	0.011
Proportion positive (P)	-	0.000
Relative abundance (I)	-	0.317
Indices from fish spotter and environmental data		
Density for positive blocks (d)	-	0.049
Proportion positive (P)	-	0.793
Relative abundance (I)	+	0.266

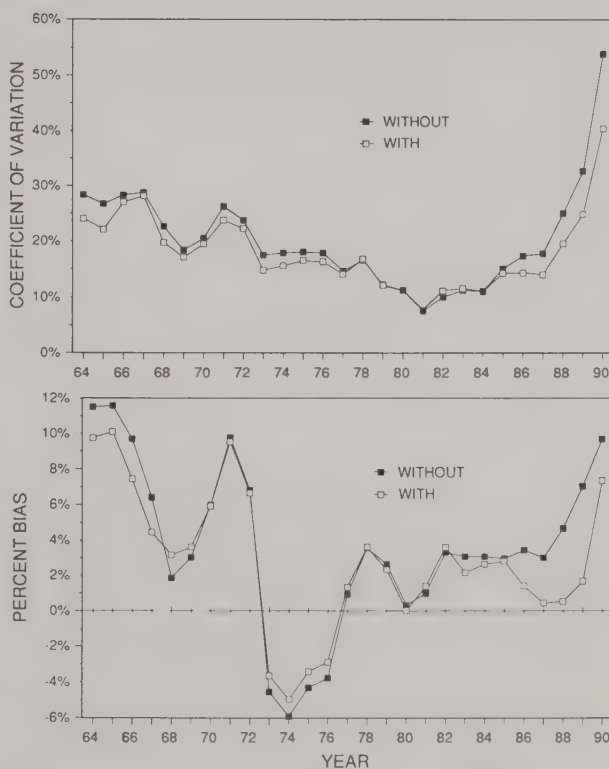


FIG. 5. Coefficients of variation (top panel) and percent bias (bottom panel) for estimates of anchovy total biomass during 1964-90 from the stock synthesis model. Coefficients of variation and percent bias were calculated by simulation for estimates made with and without fish spotter data.

on fish spotter data are cost-effective ways to improve biomass estimates used to manage the fishery for northern anchovy.

Our work extended the delta distribution models proposed by Aitchison and Brown (1957) and Pennington (1983) because both density of anchovy in positive blocks (d) and proportion positive (P) were estimated using models that allowed us to include factors (e.g. pilots) that affected estimates of density and proportion positive and increased the precision of our esti-

mates. Our approach should be applicable whenever the statistical distribution of data is distorted by excessive zeros (e.g. ichthyoplankton surveys) and affected by external factors or covariates, but care should be taken to ensure that the distribution of nonzero data is as assumed for estimation procedures (Meyers and Pepin 1990).

Future Research

The models we developed for fish spotter data may be best suited for relatively short time series because the number of parameters will increase as data are collected for more years and pilots. At some point, the number of parameters may grow large enough to make the models cumbersome and too large for small computers. Future work could usefully be directed towards reducing the number of parameters either by restricting the number of years included in the analysis or developing alternative statistical approaches.

For sufficiently long collections of fish spotter data, it may be possible and advantageous to develop time series models (Abraham and Ledolter 1983). Our approach estimated relative abundance for each year separately and may not have fully utilized the data because relative abundance of fish in adjacent years is usually similar enough to be used for making estimates (Roff 1983).

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Appendix 1. Corrections for Bias and Variance Calculations

Approximate estimates for the variance of relative abundance in each year (I in (8)) were computed by the delta method (Seber 1982):

$$(A1) \quad \text{Var}(\hat{I}) \approx A^2 [\text{Var}(\hat{d}) \hat{P}^2 + \hat{d}^2 \text{Var}(\hat{P}) + 2\hat{d}\hat{P}\text{Cov}(\hat{d}, \hat{P})]$$

where hats (ˆ) denote estimates, $\text{Var}(\hat{\cdot})$ denotes variance, I is the index of relative abundance for anchovy in each year, A is area of the anchovy stock, d is the density of anchovy in positive blocks, P is the proportion positive, and $\text{Cov}(\hat{d}, \hat{P})$ denotes the within-year covariance of estimates for density (d) and proportion positive (P). Variances for estimates of d and P were calculated as described below. The covariance term in (A1) was estimated approximately from the correlation of d and P among years and the within-year standard deviation of d and P :

$$(A2) \quad \text{Cov}(\hat{d}, \hat{P}) \approx \rho_{\hat{d}, \hat{P}} [\text{SE}(\hat{d}) \text{SE}(\hat{P})]$$

where $\rho_{\hat{d}, \hat{P}}$ denotes the correlation and $\text{SE}(\hat{\cdot})$ denotes a standard error.

Unbiased estimates of anchovy density in positive blocks (d in (5)) and proportion positive blocks (P in (7)) as well as variance estimates for d and P were calculated as described by Bradu and Mundlak (1970). The correction for bias in (5) and (7) involves a correction factor Ψ (Ψ_d for density and Ψ_p for

proportion positive). Assuming lognormal-distributed errors, the correction factor is

$$(A3) \quad \Psi = g_m \left[\frac{m+1}{2m} (\hat{\xi}^2 - \hat{\xi}_{\hat{\eta}}^2) \right]$$

where $g_m(\cdot)$ is a function described below, $\hat{\xi}^2$ is the residual variance (σ^2 in (3) for density or δ^2 in (6) for proportion positive), m is degrees of freedom for the estimate of residual variance, $\hat{\eta} = \hat{\beta}_0 + \hat{\beta}_1$ for density (d) or $\hat{\eta} = \hat{\tau}_0 + \hat{\tau}_1$ for proportion positive, and $\hat{\xi}_{\hat{\eta}}^2$ is the variance of $\hat{\eta}$.

The function $g_m(\cdot)$ in (A3) is

$$(A4) \quad g_m(t) = \sum_{p=0}^{\infty} \left[\frac{m^p (m+2p)}{m(m+2) \dots (m+2p)} \left(\frac{m}{m+1} \right)^p \frac{t^p}{p!} \right]$$

where t is the argument for the function.

Variance estimates for $\hat{d}$ in (5) and $\hat{P}$ in (7) were calculated as

$$(A5) \quad \text{Var}(\hat{\cdot}) = e^{2\hat{\eta}} \left\{ g_m^2 \left[\frac{m+1}{2m} (\hat{\xi}^2 - \hat{\xi}_{\hat{\eta}}^2) \right] - g_m \left[\frac{m+1}{m} (\hat{\xi}^2 - 2\hat{\xi}_{\hat{\eta}}^2) \right] \right\}$$

where $\text{Var}(\hat{\cdot})$ is the variance of either $\hat{d}$ or $\hat{P}$.

Appendix 2. Simulations

First, the stock synthesis model for northern anchovy was modified to include the new index of schooling biomass from fish spotter data. The original stock synthesis model for anchovy included an index of schooling biomass based on a sonar survey conducted by the California Department of Fish and Game during 1969–85 (Mais 1974; Methot 1989). Sonar and fish spotter indices both measure relative schooling biomass, so the pattern of age-specific availability of anchovy to the fish spotter index was assumed to be the same as for the sonar survey (age-specific availability patterns are used in the stock synthesis model to relate the index of schooling biomass to stock age structure). This assumption allowed us to include the new fish spotter index for anchovy with a minimum of additional programming. The fish spotter index was given the same relative weight as other types of information about abundance in the stock synthesis model.

The modified stock synthesis model was fitted to data, including the index based on fish spotter data, for 1964–90. After the model was fitted, predicted values for each type of data were calculated and stored. Standard deviations for the fit of the model to each type of relative abundance data were calculated (assuming a lognormal distribution for discrepancies between observed and predicted values).

Fifty simulated data sets with fish spotter data and 50 simulated data sets without fish spotter data were generated (the number of simulations conducted was limited by available computer time). A pseudorandom number generator, predicted values from the original fit, and standard deviations calculated as described above were used to generate relative abundance data for the simulation experiment. Age composition data were gen-

erated from predicted age compositions by assuming multinomial sampling errors. The stock synthesis model was fit to each of the simulated data sets and coefficients of variation for estimates of spawning biomass during 1964–90 were calculated

to evaluate precision. This approach to estimating coefficients of variation for spawning biomass estimates is a “parametric bootstrap” approach in the language of Efron (1982).

Mitochondrial DNA Differentiation in Western North Atlantic Populations of Haddock (*Melanogrammus aeglefinus*)

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Mitochondrial DNA polymorphism was examined in haddock (*Melanogrammus aeglefinus*) from five offshore banks in the western North Atlantic. A total of 21 genotypes were observed in an initial survey of 54 haddock with 12 restriction endonucleases. Five enzymes that revealed most of the genetic variation were used in an expanded survey from which we observed 22 genotypes among 133 haddock. Both the 12- and 5-enzyme results showed that haddock from each of the five banks comprised a mixture of divergent genotypes that could each be assigned to one of at least four groups of related genotypes differing by mean estimated divergences of 0.6–0.8%. These divergent genotypes may have arisen in past haddock populations that were more isolated than those of the present. Although genotype frequencies did not differ significantly in most pairwise comparisons of banks, two measures of genetic (dis)similarity were significantly correlated with geographic distance as well as with the presence and relative size of deepwater (>180 m) channels separating the banks. One of the genotype groups also exhibited a geographic cline in frequency of occurrence. These features indicate that gene flow among populations is restricted, and hence support the existence of discrete stocks of haddock.

Nous avons étudié le polymorphisme de l'ADN mitochondrial chez des aiglefin (*Melanogrammus aeglefinus*) provenant de cinq bancs hauturiers de la partie occidentale de l'Atlantique Nord. En tout, 21 génotypes ont été dénombrés dans une première étude effectuée sur 54 spécimens, à l'aide de 12 endonucléases de restriction. Dans une étude élargie portant sur 133 aiglefin, nous avons utilisé cinq enzymes révélant la plupart des variations génétiques et avons trouvé 22 génotypes. Les résultats obtenus à partir de 12 enzymes et ceux de l'étude à 5 enzymes indiquaient que les spécimens provenant de chacun des cinq bancs étudiés comportaient un mélange de génotypes divergents que l'on pourrait classer individuellement parmi au moins quatre groupes de génotypes apparentés dont les divergences moyennes sont évaluées entre 0,6 et 0,8 %. Ces génotypes divergents peuvent être apparus dans des populations antérieures qui étaient plus isolées que celles d'aujourd'hui. Les fréquences des génotypes ne comportaient pas de différences importantes dans la plupart des comparaisons par paire de bancs; toutefois, deux mesures de la similitude (ou de la dissimilitude) génétique avaient une corrélation étroite avec la distance géographique ainsi qu'avec la présence ou la taille relative des chenaux en eau profonde (plus de 180 m) séparant les bancs. L'un des groupes de génotypes présentait également un cline géographique dans la fréquence d'occurrence. Ces caractéristiques signifient que le flux génétique entre les populations est limité et, par conséquent, confirment l'existence de stocks séparés d'aiglefin.

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Haddock (*Melanogrammus aeglefinus*) is a commercially exploited gadid species occurring on both sides of the North Atlantic. In the east it ranges from the White and Kara seas south to the Bay of Biscay. In the west its range is from the Strait of Belle Isle south to Cape Hatteras (Scott and Scott 1988); however, the major harvested aggregations are more restricted in distribution, occurring on the southern Grand Bank, the southern Gulf of St. Lawrence, across the Scotian Shelf into the Bay of Fundy, and on the northern portion of Georges Bank. These aggregations have been fished heavily throughout this century (e.g. Zwanenburg 1989), generating an interest in designing fisheries management strategies to ensure their long-term viability. A major goal of haddock management has been the delineation of fishing areas such that their boundaries correspond to those of independently reproducing populations (see Halliday and Pinhorn 1990 for a detailed review).

The delineation of haddock populations is complicated by seasonal differences in spatial distributions. Most haddock spawn in discrete aggregations on offshore banks (Fig. 1) during late winter or spring, but at other times they may be much more dispersed (Needler 1929, 1930). Haddock less than 4 yr old tend to remain on or around the offshore banks on a year-round basis. Haddock spawning on the eastern Scotian Shelf are thought to migrate into the southern Gulf of St. Lawrence during the warmer months of the year and return to the offshore banks during the fall (Needler 1930; McCracken 1956).

To date, inferences regarding the population structure of haddock have been based on three sorts of data: demographic differences (growth rates, year-class composition of catches, and population-size fluctuations), meristic differences (vertebral counts), and tagging data. A series of studies using various combinations of these methods have supported the existence of

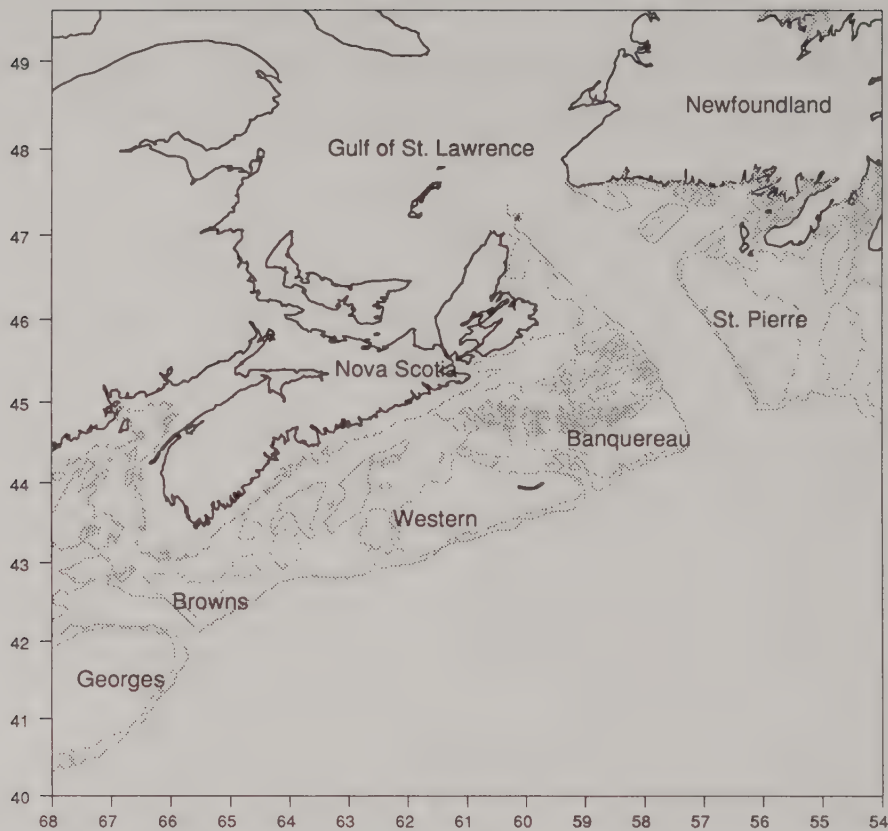


FIG. 1. Map of the Northwest Atlantic showing the banks from which haddock were collected for the present study. Exact locations and sample numbers are given in Table 1.

as few as three and as many as 11 discrete stocks of haddock in the western North Atlantic (Needler 1930; Vladykov 1935; Martin 1953; Templeman 1953; Grosslein 1962). Current management practice recognizes five units: Grand Banks, St. Pierre Bank, Eastern Scotian Shelf (including Banquereau Bank) and Southern Gulf of St. Lawrence, Western Scotian Shelf, and Georges Bank.

Although they are supported by an extensive body of data, the biological significance of the five management units above remains unclear. Demographic and meristic differences between geographic areas may be partially or even entirely the result of locally varying environmental factors, and hence do not unambiguously signal reproductive isolation. Similarly, tagging data, although very useful for tracking the movements of individuals, do not reveal whether migrants from one area to another are successfully reproducing.

Genetic data provide the most stringent test of reproductive isolation. Genetic differences among geographic areas can only occur if gene flow (migration followed by successful reproduction) is of very low magnitude, or if genetic variants are subject to strong and locally varying selective pressures. Consequently, genetic differences involving traits that are not, or only weakly, selected offer unambiguous evidence of reproductive isolation. Although genetic data have not figured in the current view of haddock population structure in the western North Atlantic, recent evidence that genetically discrete populations of haddock occur in the eastern North Atlantic (Jamieson and Birley 1988) suggests that the same might be true in the western North Atlantic.

Mitochondrial DNA (mtDNA) has attributes which uniquely suit it to the study of genetic differentiation within species. Its application to fishery biology has been reviewed thoroughly (Ferris and Berg 1987; Ovenden 1990; see also Wilson et al. 1985; Avise et al. 1987; Moritz et al. 1987 for more general reviews). Here we merely note certain key points. In homeothermic vertebrates at least, mtDNA evolves more rapidly than nuclear DNA (Brown et al. 1979; Wilson et al. 1985). As a consequence of being maternally inherited in a functionally haploid manner, effective gene flow for mtDNA is expected to be 25% that of nuclear DNA when migration of females and males occurs at an equal rate (Birky et al. 1983, 1989). As a result of these factors, genetic variation among geographic localities is often more dramatic for mtDNA than for nuclear DNA or nuclear gene products, such as isozymes. Finally, because mtDNA evolves without recombination, phylogenetic inferences can be drawn from genetic variation, allowing some interpretation of the historical processes that have influenced the development of modern populations (Avise et al. 1987).

As an initial step in the genetic characterization of western North Atlantic populations of haddock, we present the result of a survey of mtDNA variation in haddock sampled from five geographic areas representing four of the five currently defined haddock stocks. To ensure that the haddock we studied could be unambiguously assigned to particular stocks, we sampled directly from the offshore banks where spawning occurs, either immediately before or after (one sample only) the major period of spawning. Our results provide indications not only of cur-

rent, but also of past population divisions in this species, and of the importance of deepwater channels as barriers to dispersal.

Materials and Methods

Haddock were collected from five offshore banks (Table 1; Fig. 1). Ovaries or livers were removed in the field and kept on wet ice for a maximum of 4 d before being processed. mtDNA was extracted from 5–12 g of tissue using the modified Chapman and Powers (1984) method described by Bentzen et al. (1988) with one additional modification: the sucrose cushion was eliminated from the first centrifugation step.

Restriction endonuclease digests of mtDNA were carried out under conditions specified by the vendor (Boehringer Mannheim Canada). DNA fragments were separated in 1% w/v horizontal agarose gels containing boiled RNase A ($0.2 \mu\text{g} \cdot \text{mL}^{-1}$). The RNase served to eliminate RNA that might otherwise have obscured DNA fragments of 0.5 kilobase (kb) or less. DNA bands were visualized with EtBr staining, and fragment sizes were estimated by reference to a 1-kb DNA ladder (Bethesda Research Laboratories) run concurrently on each gel.

Initially, 17 restriction enzymes with five or six base pair (bp) recognition sequences were tested on haddock mtDNA. Five enzymes (*KpnI*, *BglI*, *SalI*, *BamHI*, *ClaI*) cut the DNA once or not at all, and were not used further. The remaining 12 enzymes, *AvaII*, *SylI*, *HaeII*, *BstEII*, *EcoRI*, *ScaI*, *HincII*, *HindIII*, *PvuII*, *PstI*, *XbaI*, and *EcoRV*, were used to assay the mtDNA of 54 haddock. Of the 12, *AvaII*, *SylI*, *HaeII*, *BstEII*, and *EcoRI*, were also used to assay mtDNA from an additional 79 haddock.

Sequence divergences among genotypes in the 12-enzyme data set were estimated from fragment data using the Nei and Li (1979) method and the REAP software package distributed by I. Kornfield (University of Maine, Orono, ME). Nucleotide diversity was calculated according to Nei and Li (1979). The nucleon diversity index of mtDNA haplotypes was estimated according to Nei and Tajima (1981).

The heterogeneity in the frequencies of mtDNA genotypes among samples was analyzed by comparing calculated chi-square values with distributions of the chi-square statistic generated by randomizations of the data using a Monte Carlo technique (Roff and Bentzen 1989). This procedure avoids problems created by the occurrence of many cells with low expected frequencies, such as occurs with rare mtDNA genotypes and small sample sizes. By this method the accuracy of the estimate of Type 1 error depends only on the number of randomizations performed (usually 1000). The randomizations were performed using the MONTE program in REAP.

Nei's (1975, 1978) measure of genetic distance (D) was used to compare samples from different banks in the 5-enzyme data

set. In this analysis, haplotypes were treated as alleles in Nei's formulation. The significance of the relationship between D and geographic distance between banks was tested using a Monte Carlo based approach. Due to the novelty of this approach, a brief discription is included with the results.

Results

12-Enzyme Data Set

The 12 enzymes revealed 62–65 restriction sites per individual and a total of 79 sites overall. Two enzymes, *EcoRV* and *PstI*, revealed no restriction site variation among the 54 haddock mtDNAs examined. The remaining 10 enzymes each detected two to eight genotypes, differing from each other by site gains or losses (Table 2). The size of the haddock mitochondrial genome, estimated to be $16.6 \text{ kb} \pm 0.5 \text{ kb}$ (SD) by summing restriction fragments, was the same in all but two fish. Those two haddock, from Western Bank, were heteroplasmic. They each bore mitochondrial genomes of two different sizes, the 16.6-kb size shared by other haddock as well as a larger form, 16.9 kb in one fish, and 17.8 kb in the other.

The 10 enzymes that detected restriction site variation collectively defined 21 composite genotypes (haplotypes), 15 of which were observed in single individuals (Table 3). Of the remaining six haplotypes, the three most common occurred in all areas whereas the other three were found at only some of the locations sampled. The nucleon diversity index (h , Nei and Tajima 1981) ranged from 0.83 to 0.93 within population samples and was 0.87 for all samples combined (Table 3). The maximum estimated sequence divergence among haplotypes was 1.29% (Table 4), and overall sequence diversity (π) among individuals was 0.61%.

Pairwise sequence divergence estimates were used to cluster haplotypes by UPGMA analysis (Fig. 2). Except for three haplotypes (representing four haddock) the various haplotypes grouped in four clusters (A–D).

5-Enzyme Data Set

Five enzymes that revealed most of the genetic variation observed in the survey above, *AvaII*, *SylI*, *HaeII*, *BstEII*, and *EcoRI*, were used to assess haplotype frequencies among an additional 79 individuals for a total of 133 haddock, representing 20–32 fish from each of the five banks (Table 1). In this larger group of fish the five enzymes defined 22 haplotypes, 12 of which were observed in single individuals (Table 5). The four most common haplotypes were observed in haddock from all five banks. With this suite of enzymes, $h = 0.73$ – 0.85 within population samples and 0.79 overall (Table 5).

TABLE 1. Sampling locations for haddock.

Location	Latitude	Longitude	Date (d/mo/yr)	Tissue	<i>n</i>
Georges Bank	41°53'N	66°37'W	06/03/89	Roe	8
Georges Bank	42°13'N	66°20'W	06/06/89	Ovary/liver	20
Browns Bank	42°24'N	64°59'W	28/02/89	Roe/liver	11
			12/02/90	Roe	21
Western Bank	43°20'N	62°07'W	20/03/89	Roe	20
Banquereau Bank	44°05'N	58°30'W	19/03/89	Roe	12
Banquereau Bank	44°15'N	59°00'W	06/04/89	Roe	15
Banquereau Bank	44°26'N	57°54'W	13/05/89	Roe	6
St. Pierre Bank	45°35'N	54°50'W	19/03/89	Roe	20

TABLE 2. Estimated sizes (in base pairs) of haddock mtDNA fragments resulting from digestion with restriction endonucleases. Entries in parentheses refer to fragments not seen on the gel, but which were inferred from fragment changes in other genotypes. For each enzyme, letters refer to the different genotypes revealed, with "A" denoting the most common genotype.

<i>Ava</i> II	A	B	C	D	E	F	G	H	<i>Sty</i> I	A	B	C	D	E
6 760									4 250	—		—	—	—
5 800		—							2 600	—	—		—	
5 600							—		2 260		—			
5 200	—	—	—	—	—	—		—	2 200	—	—	—	—	—
3 860								—	2 100			—		
3 500	—			—					1 920		—			
2 400			—						1 670				—	
2 300					—				1 500	—	—	—	—	—
2 160	—		—	—		—			1 370	=	=	=	—	=
1 950					—	—			1 330					—
1 800								—	1 270					—
1 750	—	—	—	—	—	—	—	—	830	—	—	—	—	—
1 500	—	—	—	—	—	—	—	—	760	—	—	—	—	—
1 480						—			620	—	—	—	—	—
1 170	—	—	—	—	—	—	—	—	520	—	—	—	—	—
950			—						500			(—)		
850					—				306	—	—			—
620	—	—	—	—	—	—	—	—						
610					—									
390	—	—	—		(—)	—	—	—						
370				—										
250	—	—	—	—	(—)	—	—	—						
<i>Hae</i> II	A	B	C	D		<i>Bst</i> EII	A	B	C		<i>Eco</i> RI		A	B
10 900			—			12 000	—				8 300		—	=
6 600	—	—				9 300		—			5 300			
5 910		—				7 600			—		2 950		—	
5 800			—			5 000								
5 100				—		4 700								
5 000	—			—		4 600	—	—	=					
4 350	—	—		—		2 650		—						
1 500				—										
910	—			—										
<i>Sca</i> I	A	B	C			<i>Hinc</i> II	A	B			<i>Hind</i> III		A	B
6 600		—				3 500	=	=			9 100		—	
4 300	—	—	—			3 350	—				6 400			—
4 200	=	—	—			2 920		—			5 800		—	—
2 800			—			1 380	—	—			2 700			—
2 400	—		—			1 270	—	—			950		—	—
1 500	—	—	—			1 030	—	—			870		—	—
1 400			—			950	—	—						
						850	—	—						
						420		—						
						350	—	—						
<i>Pvu</i> II	A	B				<i>Pst</i> I	A				<i>Xba</i> I		A	B
8 700	—	—				5 250	—				9 600		—	
4 900		—				4 700	—				4 900		—	—
4 200	—					4 000	—				4 850			=
1 750	—	—				1 620	—				1 350		—	—
1 320	—	—				910	—				1 320		—	
520	—										1 300			—
<i>Eco</i> RV	A													
9 600	—													
8 900	—													

The twenty-two 5-enzyme haplotypes were grouped in a parsimony network based on inferred restriction site losses and gains among genotypes (Fig. 3). This analysis revealed several groups of related haplotypes. Two of these groups, III and IV, are relatively well defined and correspond to the C and D clusters in the UPGMA phenogram (Fig. 2). Groups I and II are more loosely defined and include rare genotypes which differ by more than a single site loss or gain.

A Monte Carlo analogue of the chi-square test (Roff and Bentzen 1989) was used to test for heterogeneity in haplotype frequencies among samples in a series of analyses. Except where noted below, all probability estimates (*P*) are based on 1000 randomization replicates. Initially, we tested for heterogeneity among samples taken from the same bank on different dates. Three banks were sampled on two or three dates, while the remaining banks were sampled on only one occasion

TABLE 3. 12-enzyme haplotypes of haddock. The letter designations refer to single enzyme genotypes in the order presented in Table 2; "h" denotes values of Nei and Tajima's (1981) nucleon diversity index.

	Haplotype	Location					
		Georges	Browns	Western	Banquereau	St. Pierre	Total
I	AAABAAAAAAAA	3	3	5	4	2	17
II	ACCAAAAAAAAA	1	3	1	1	1	7
III	BBBAAAAAAAAA	1	1	2	1	3	8
IV	AABCAAAAAAAAA	0	0	1	2	0	3
V	AABAAAAAAAAA	1	0	1	0	0	2
VI	CABCBAAAAAAAA	0	1	0	0	1	2
VII	EADAAAAAAAAA	1	0	0	0	0	1
VIII	BCCAAAAAAAAA	1	0	0	0	0	1
IX	FCCAAAAAAAAA	0	1	0	0	0	1
X	BBBBAAAAAAAAA	0	1	0	0	0	1
XI	DABAAAAAAAAA	0	1	0	0	0	1
XII	DAABAAAAAAAA	0	0	1	0	0	1
XIII	BBBABAAAAAAAA	0	0	1	0	0	1
XIV	ADBAAABAAAAA	0	0	0	1	0	1
XV	AABAABAAAAAA	0	0	0	1	0	1
XVI	BAABAAAAAAAA	0	0	0	1	0	1
XVII	AABCBCAABAAA	0	0	0	1	0	1
XVIII	BBBAAAAAAAABA	0	0	0	0	1	1
XIX	AEBAAAAAAAAA	0	0	0	0	1	1
XX	ACCAAAABAAAA	0	0	0	0	1	1
XXI	HBBAAAAAAAAA	0	0	0	0	1	1
Total		8	11	12	12	11	54
h		0.8928	0.8909	0.8333	0.8939	0.92731	0.8672

TABLE 4. Estimated percent sequence divergence (below diagonal) and standard errors (above diagonal) between haplotypes.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI
I	—	0.69	0.81	0.49	0.46	0.78	0.87	0.90	0.88	0.74	0.66	0.46	0.86	0.62	0.54	0.58	0.67	0.85	0.56	0.71	0.86
II	0.76	—	0.84	0.59	0.52	0.85	0.97	0.60	0.56	0.90	0.70	0.83	0.90	0.60	0.59	0.90	0.76	0.89	0.54	0.23	0.89
III	0.67	0.72	—	0.73	0.67	0.83	0.89	0.61	0.89	0.32	0.82	0.93	0.28	0.79	0.73	0.57	0.87	0.28	0.74	0.87	0.58
IV	0.40	0.52	0.46	—	0.28	0.62	0.91	0.83	0.80	0.76	0.54	0.67	0.78	0.51	0.39	0.76	0.47	0.77	0.43	0.62	0.78
V	0.35	0.38	0.31	0.15	—	0.68	0.87	0.78	0.76	0.75	0.47	0.66	0.73	0.43	0.28	0.75	0.55	0.73	0.33	0.56	0.73
VI	0.72	0.84	0.68	0.30	0.46	—	0.98	0.92	0.90	0.86	0.81	0.90	0.79	0.80	0.74	0.86	0.68	0.87	0.75	0.87	0.87
VII	0.68	0.95	0.75	0.79	0.65	1.01	—	0.94	0.91	0.94	0.97	0.97	0.94	0.96	0.92	0.83	1.03	0.93	0.92	0.99	0.92
VIII	0.92	0.16	0.56	0.69	0.54	0.91	0.89	—	0.66	0.69	0.91	1.02	0.68	0.84	0.84	0.69	0.96	0.67	0.80	0.63	0.85
IX	0.91	0.16	0.78	0.68	0.53	0.90	0.88	0.23	—	0.95	0.88	0.98	0.94	0.82	0.81	0.95	0.94	0.93	0.77	0.60	0.93
X	0.47	0.92	0.20	0.56	0.51	0.79	0.95	0.77	0.98	—	0.88	0.88	0.43	0.85	0.80	0.46	0.90	0.42	0.81	0.92	0.67
XI	0.45	0.48	0.44	0.25	0.10	0.59	0.80	0.68	0.66	0.64	—	0.46	0.87	0.63	0.55	0.88	0.72	0.86	0.57	0.73	0.86
XII	0.10	0.86	0.80	0.50	0.45	0.84	0.83	1.06	1.04	0.60	0.35	—	0.98	0.77	0.72	0.74	0.82	0.97	0.73	0.85	0.97
XIII	0.84	0.88	0.15	0.62	0.47	0.52	0.91	0.72	0.94	0.37	0.60	0.97	—	0.84	0.79	0.63	0.83	0.39	0.80	0.91	0.65
XIV	0.62	0.56	0.59	0.40	0.26	0.72	0.94	0.72	0.71	0.79	0.36	0.72	0.74	—	0.51	0.85	0.69	0.83	0.53	0.64	0.84
XV	0.52	0.53	0.47	0.31	0.15	0.64	0.80	0.70	0.69	0.68	0.25	0.62	0.63	0.41	—	0.80	0.58	0.78	0.44	0.63	0.79
XVI	0.16	0.93	0.51	0.56	0.51	0.79	0.62	0.77	0.98	0.31	0.64	0.29	0.68	0.79	0.68	—	0.90	0.62	0.81	0.92	0.81
XVII	0.93	1.04	0.97	0.47	0.66	0.46	1.29	1.21	1.19	1.10	0.76	1.03	0.80	0.91	0.73	1.10	—	0.90	0.64	0.77	0.91
XVIII	0.82	0.87	0.15	0.61	0.46	0.84	0.89	0.71	0.92	0.36	0.59	0.95	0.31	0.73	0.62	0.67	1.15	—	0.79	0.90	0.64
XIX	0.51	0.43	0.47	0.30	0.15	0.61	0.81	0.60	0.59	0.67	0.25	0.61	0.62	0.42	0.30	0.67	0.80	0.61	—	0.58	0.80
XX	0.85	0.10	0.81	0.62	0.47	0.94	1.03	0.26	0.26	1.02	0.57	0.95	0.97	0.64	0.63	1.02	1.14	0.95	0.52	—	0.91
XXI	0.73	0.78	0.16	0.51	0.37	0.74	0.81	0.72	0.84	0.36	0.51	0.86	0.31	0.64	0.52	0.67	1.02	0.31	0.53	0.86	—

(Table 1). The heterogeneity in composite genotype frequencies was not significant for within-bank comparisons of samples drawn from Georges, Banquereau, and Browns banks ($P \approx 0.37$, 0.36, and 0.12, respectively). In all further analyses, samples from the above three banks were aggregated by bank.

The heterogeneity in composite genotype frequencies among the five banks was not significant ($P \approx 0.60$). Heterogeneities in single-enzyme genotype frequencies were also not significant for each of the five enzymes tested in turn, although the probability level associated with *StyI* genotypes approached statistical significance ($P \approx 0.84$, 0.076, 0.53, 0.31, and 0.29 for

AvaII, *StyI*, *HaeII*, *BstEII*, and *EcoRI*, respectively; 10 000 randomizations performed for *StyI*).

Pairwise tests for heterogeneity were performed for all combinations of two banks, both for 5-enzyme composites and *StyI* single-enzyme genotypes. None of the comparisons based on composite genotypes revealed significant heterogeneity; however, a spatial trend in the values of probability estimates emerged. Estimates of P tended to be relatively high for comparisons of neighbouring banks and lower for those further apart (Table 6). Estimates of P based on *StyI* genotype frequencies tended to be lower than corresponding estimates based on com-

Percent Sequence Divergence

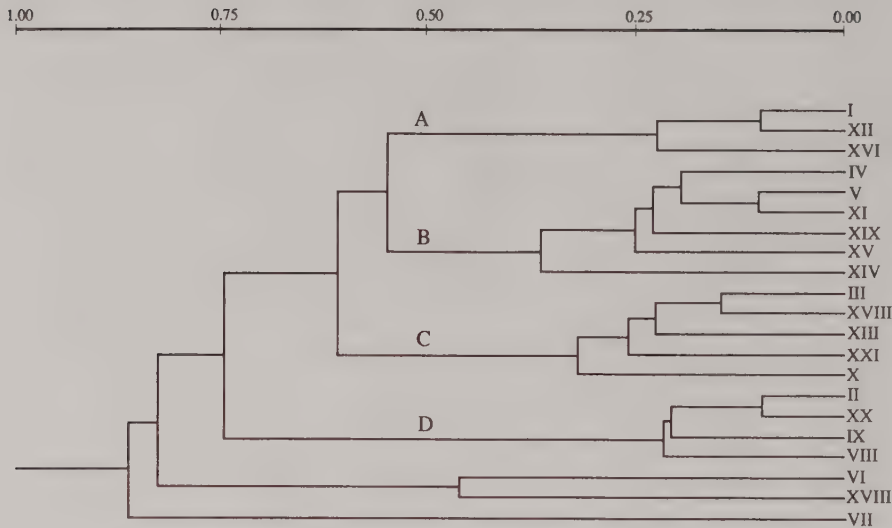


FIG. 2. UPGMA phenogram showing the relationships of haddock mtDNA genotypes based on sequence divergence estimates in Table 4. Letters refer to clusters of genotypes discussed in the text.

TABLE 5. 5-enzyme haplotypes of haddock.

Haplotype	Location					Total
	Georges	Browns	Western	Banquereau	St. Pierre	
AAABA	10	14	10	14	6	54
ACCAA	5	5	3	3	4	20
BBBAA	2	1	3	4	4	14
AABAA	4	2	1	3	1	11
AABCA	2	1	1	2	0	6
CABCB	0	1	0	3	1	5
AAAAA	3	1	0	0	0	4
BCCAA	1	1	0	0	1	3
AEBAA	0	0	0	0	2	2
HBBA	0	1	0	0	1	2
BAABA	0	0	0	1	0	1
AABCB	0	0	0	1	0	1
GBBAA	0	0	0	1	0	1
FCCAA	0	1	0	0	0	1
BBBBA	0	1	0	0	0	1
DABAA	0	1	0	0	0	1
DAABA	0	0	1	0	0	1
BBBAB	0	0	1	0	0	1
ADBAA	0	0	0	1	0	1
EADAA	1	0	0	0	0	1
ICCAA	0	1	0	0	0	1
JAABA	0	1	0	0	0	1
Total	28	32	20	33	20	133
<i>h</i>	0.8254	0.7943	0.7316	0.7973	0.8526	0.7947

posite genotypes, but showed a similar spatial trend (Table 6). Probability estimates for three of the pairwise comparisons for *StyI*, Georges/St. Pierre, Browns/St. Pierre, and Banquereau/St. Pierre, approached significance or were marginally significant ($P \approx 0.04$, 0.07 , and 0.04 , respectively). Regressions of pairwise P estimates on geographic distance between sampling locations were significant for both composite ($p < 0.05$,

$R^2 = 0.40$) and single-enzyme *StyI* genotypes ($p < 0.05$, $R^2 = 0.44$) (Fig. 4).

Nei's genetic distance (D) was calculated for all possible pairs of banks, using the frequencies of composite genotypes and single-enzyme *StyI* genotypes as the frequencies of alleles in alternate comparisons. Both measures of D increased with increasing geographic distance. The significance of the

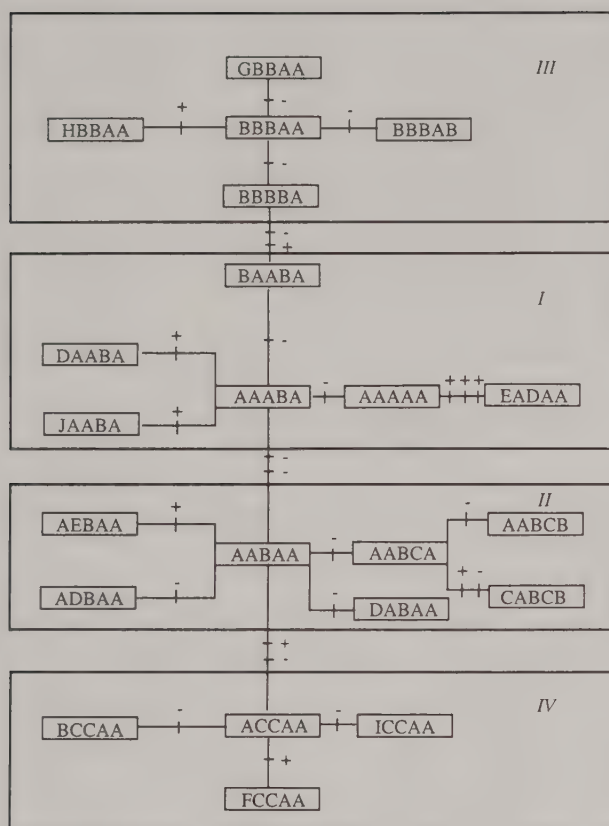


FIG. 3. Parsimony network of 5-enzyme haplotypes for Northwest Atlantic haddock. The network was constructed by connecting related haplotypes based on the minimum number of inferred site gains (+) and losses (-) relative to the most common genotype, AAABA. Roman numerals refer to clusters defined by the boxes and are discussed in the text.

observed correlation between geographic and genetic distance was tested by repeated randomization of the observed 5-enzyme haplotype frequencies (Table 5) while conserving the marginal totals to reflect sampling intensities and overall genotype frequencies. For each randomization, genetic distances between all sites were recalculated and the resulting correlation between D and geographic distance tested against that observed for the nonrandomized data set. The number of times the test statistic exceeds the nonrandomized statistic from a total of 10 000 randomizations gives the probability of the original correlation occurring by chance alone. The distribution of the randomized test statistic from 1000 randomizations (Fig. 5) shows no evidence of bias in the randomization algorithm. These analyses showed that for the composite genotypes the observed corre-

lation with geographic distance ($r = 0.70$) occurred in only 240 of 10 000 randomizations (2.4%); hence, $P \approx 0.024$ (Fig. 6). For the single-enzyme *Sst*I genotype the observed correlation ($r = 0.48$) was exceeded in 140 of 1000 randomizations ($P \approx 0.14$).

Two of the five locations sampled are separated from the others by deepwater channels. Georges Bank is separated from banks on the Scotian Shelf by the Fundian Channel, and St. Pierre Bank is separated from the others by the much broader and deeper Laurentian Channel (Fig. 1). To test the influence of channels on spatial trends in frequencies of haddock mitochondrial genotypes, a "channel factor" was used in place of geographic distance. This factor incorporates both the width and depth (width at 360-m isobath $\times$ maximum depth) summed across the number of intervening channels. The correlation ($r = 0.80$) between channel factor and D for composite genotypes (Fig. 7) was also tested using the randomization process outlined above. In this case the observed correlation was exceeded in 2.8% of the randomizations ($P \approx 0.028$). The correlation between D for *Sst*I genotypes and geographic distance ($r = 0.88$) was exceeded by chance in 1.8% of all cases ($P \approx 0.018$).

One other trend was evident in the data. Haplotype group III identified in the parsimony network (Fig. 3) exhibited a geographic cline in frequency of occurrence (Fig. 8). This relationship is marginally significant in that the correlation ($r = 0.89$) was exceeded in 5% of 1000 randomizations ($P \approx 0.05$).

Discussion

We initially tested 12 enzymes on 8–12 haddock from each of the five banks sampled. This survey revealed extensive polymorphism, a point demonstrated by the nucleon diversity index, for which $h = 1$ is the maximum value attainable. For haddock in this survey, $h = 0.87$, a value close to that obtained for Atlantic herring (*Clupea harengus*) sampled in the same general geographic area ($h = 0.91$; Kornfield and Bogdanowicz 1987) but markedly higher than that for a similar gadid species, Atlantic cod (*Gadus morhua*), from around Newfoundland ($h = 0.36$; Carr and Marshall 1991). Nucleotide diversity (π) was also higher among haddock mtDNAs than among those of Newfoundland cod ($\pi = 0.59$ and 0.18% , respectively; Carr and Marshall 1991). The methodology used by Carr and Marshall differed from that used in this study: they directly sequenced a 298-bp region of the mitochondrial gene coding for cytochrome *b*. Although it is possible that the difference in methodology could account for the greater magnitude of genetic variation observed in haddock, this is unlikely for several reasons. First, despite different technical approaches, the two studies are similar in sampling effort at the molecular level, since the 62–65 restriction sites per individual that we assayed correspond to an average of 338 bp surveyed for sequence varia-

TABLE 6. Estimates of P for pairwise comparisons of banks. Values are for tests based on 5-enzyme composite genotypes (above diagonal) and for tests based on *Sst*I single-enzyme genotypes (below diagonal).

Location	Georges	Browns	Western	Banquereau	St. Pierre
Georges	—	0.87	0.54	0.31	0.22
Browns	0.83	—	0.93	0.64	0.70
Western	0.42	0.50	—	0.79	0.63
Banquereau	0.35	0.22	0.88	—	0.38
St. Pierre	0.04	0.07	0.31	0.04	—

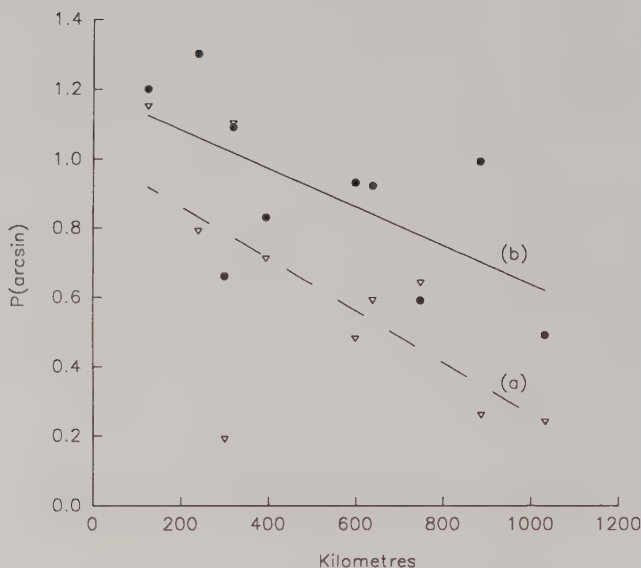


FIG. 4. Relationship between distance separating Northwest Atlantic banks and the probability of heterogeneity of haddock genotype frequencies between those banks: (a) Composite; (b) *StyI*. Probabilities were transformed (square root arcsin) and the line represents a fitted linear model.

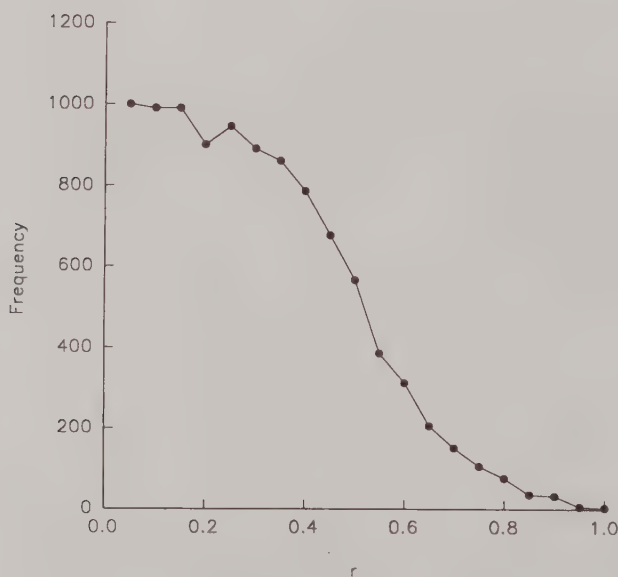


FIG. 5. Distribution of correlation coefficients resulting from 10 000 randomizations of the 5-enzyme genotype data given on Table 5. A brief description of the randomization procedure is given in the text.

tion. Second, a low level of mtDNA polymorphism was also observed in a conventional restriction enzyme survey of cod from the Grand Banks and North Sea (Smith et al. 1989). Third, Carr and Marshall (1991) observed greater variation in cytochrome *b* sequences of Norwegian cod ($h = 0.88$, $\pi = 0.47\%$), an observation consistent with the results of a recent restriction enzyme survey of Norwegian cod mtDNAs (Dahle 1991). This third point suggests that the low level of variation in the cytochrome *b* sequences of Newfoundland cod

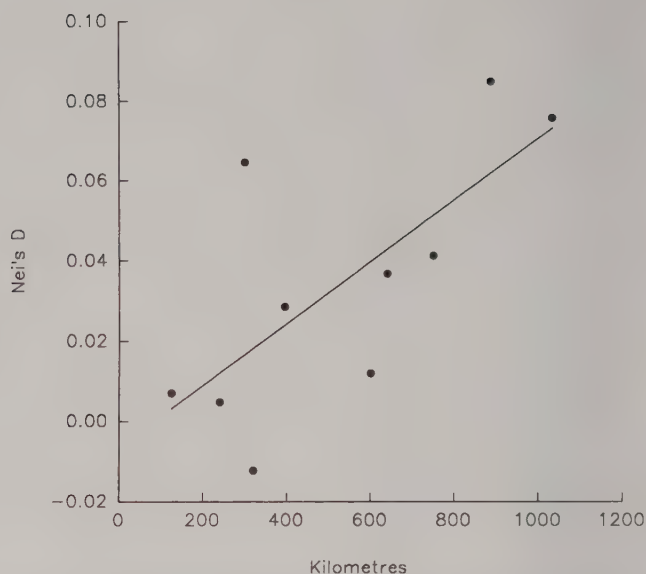


FIG. 6. Relationship between distance separating Northwest Atlantic banks and Nei's *D* for haddock mtDNAs. Values of *D* were obtained by treating 5-enzyme genotypes as alternate alleles. The line represents a fitted linear model.

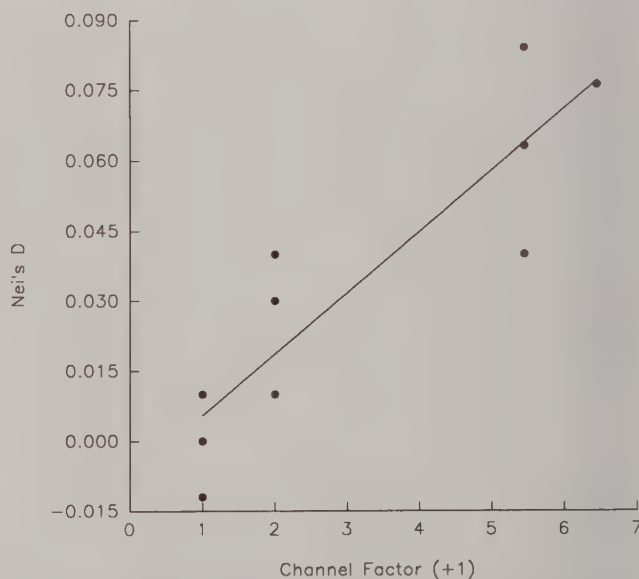


FIG. 7. Relationship between channel factor (CF) and Nei's *D* for haddock mtDNAs. CF is defined as width at 360-m isobath $\times$ maximum depth of the Laurentian and Fundian channels, respectively. Values of *D* were derived as in Fig. 6. The line represents a fitted linear model.

is more likely a product of population-level effects rather than an intrinsic lack of variation in this particular DNA sequence.

The difference in the magnitudes of variation exhibited by the mtDNAs of haddock and cod in the western North Atlantic is intriguing. Assuming that haddock and cod mtDNAs evolve at similar rates, the difference in the extent of genetic variation signals a difference in the long-term effective population sizes (N_e). Two factors that may contribute to N_e s substantially lower than suggested by current census estimates are historical bottle-

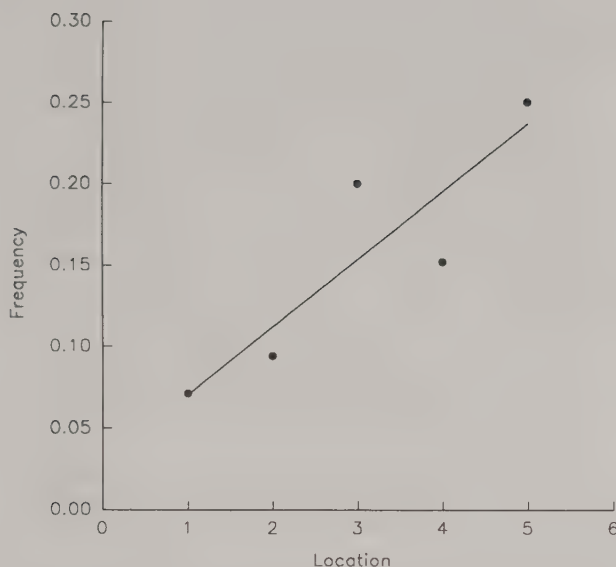


FIG. 8. Geographic cline in frequencies of haplotype group III (Fig. 3). The linear model was fitted using square root arcsin transformed frequencies. Location: 1 = Georges Bank; 2 = Browns Bank; 3 = Western Bank; 4 = Banquereau Bank; 5 = St. Pierre Bank.

necks in population size and high variance in reproductive success such as may occur in species with very high fecundities (Avise et al. 1984; Wilson et al. 1985). The second factor reduces N_e by channelling mitochondrial lineages through relatively few females in each generation.

The possibility that cod may have experienced more severe bottlenecks than haddock during Pleistocene glacial advances cannot be ruled out, but seems unlikely for two reasons. First, the current range of cod extends much further north than that of haddock (Scott and Scott 1988), indicating a greater tolerance of low temperatures during the life cycle. Second, of the two species, cod are considered more mobile, an attribute probably related to the more piscivorous nature of their diet.

The second possibility, that cod exhibit higher reproductive variance, is almost equally problematic. Haddock and cod have similarly high fecundities, and of the two species, haddock show relatively greater year to year variance in recruitment success. The number of age 1 fish estimated for Eastern Scotian Shelf haddock averaged $38 \times 10^6 \pm 29 \times 10^6$ (SD) for the period 1948–86 (Zwanenburg and Fanning 1988), while for cod in the same area, recruitment at age 1 averaged $85 \times 10^6 \pm 32 \times 10^6$ (SD) (Fanning et al. 1988). Nevertheless, the possibility that cod experience greater variance in female to female reproductive success within years cannot be discounted.

Of the 12 enzymes initially evaluated, only five revealed variant genotypes in more than one individual (Table 3). The same five enzymes were the only ones that revealed characters that were informative in the phylogenetic sense, that is, variants that were present in more than one composite genotype. Since in this study our primary aim was to evaluate the relative frequencies of well-defined haplotypes, we focused our analysis on these five enzymes (Table 5) in an expanded survey of 20–32 haddock from each bank.

Both the 12- and 5-enzyme data sets showed that haddock sampled from each of the five banks comprised a mixture of relatively divergent genotypes that could each be assigned to

one of at least four groups (Fig. 2 and 3). The three most common genotypes (AAABA, ACCAA, and BBBAA; Table 5) each belong to a separate genotype group. These three haplotypes differ from each other by four mutation steps (gains or losses of restriction sites), corresponding to estimated sequence divergences of 0.6–0.8% (Table 4).

The genotype clusters are not statistically supported as discrete lineages. Note, for instance, that the standard errors on the sequence divergence estimates are of similar magnitude to the divergence estimates themselves (Table 4). Similarly, a bootstrap analysis of parsimony trees generated from inferred site differences among haplotypes using the BOOT program in PHYLIP (Felsenstein 1985) did not reveal any statistically significant branches (data not shown). A statistically robust determination of the number of mitochondrial clades present in western Atlantic haddock will require more genetic data, either through the use of more restriction enzymes or via direct sequence analysis of the mtDNA haplotypes.

Despite the lack of statistical support from the genetic data, however, circumstantial evidence suggests that the genotype clusters represent at least two distinct mitochondrial lineages. This evidence comes from a geographic cline in the frequency of group III haplotypes (Fig. 8), which suggests a distinct evolutionary history for this haplotype group (see below).

This apparent multimodality of mitochondrial lineages in modern populations of haddock could be a consequence of random, stochastic lineage sorting operating within haddock populations (Avise et al. 1984; Slatkin and Hudson 1991; see also Saunders et al. 1986). Alternatively, the divergent mitochondrial lineages could stem from some period in the past when populations of haddock were more isolated from each other than they are at present. In this model, modern populations comprise mixtures of matrilineal descendents from these earlier, isolated populations. This latter possibility is supported by similar data from other species. Current populations of at least three other species that occur in the western North Atlantic, American lobster (*Homarus americanus*), Atlantic herring, and rainbow smelt (*Osmerus mordax*) comprise mixtures of two distinct mitochondrial clades (Kornfield and Bogdanowicz 1987; Kornfield and Moran 1989; Baby et al. 1992; I. Kornfield, Department of Zoology, University of Maine, Orono, ME, pers. comm.). Moreover, the estimated sequence divergences that characterize the clades in lobster, herring, and smelt (0.9, 1.0, and 0.8%, respectively) are similar to those that distinguish the presumptive clades in haddock (0.6–0.8%). This concordance suggests that mitochondrial lineages in haddock, lobster, herring, and smelt may share a similar history of isolation and differentiation in the past followed by mixing in modern populations.

It is tempting to speculate about the age and nature of the event(s) that might have led to the phylogenetic divisions in the mtDNAs of these disparate species. If the divergence rate in these species is not greater than the “rapid” rate of 2% per million years calibrated in various homeothermic vertebrates (Wilson et al. 1985), then the phylogenetic splits date from at least 350 000 – 450 000 yr B.P., an age that corresponds approximately to the beginning of the Illinoian glaciation. If the phylogenetic divisions in the mtDNAs of these marine species were initiated during or prior to the Illinoian glaciation, then the alternative clades must have persisted through major climatic fluctuations, including the Sangamon interglacial and the subsequent Wisconsin glaciation.

Although populations of haddock may have been well differentiated genetically at times in the past, this is not true of modern populations. We were not able to detect statistically

significant differences in composite genotype frequencies among any of the five banks that we sampled, although three between-bank comparisons of single-enzyme *Syl* genotype frequencies approached significance or were marginally significant (Table 6). Nevertheless, our results indicate that gene flow among populations is restricted. Two measures of genetic (dis)similarity, the probability values (P) obtained from our chi-square randomizations and Nei's D , both exhibited marked trends in pairwise comparisons of populations (Fig. 4 and 6). Both measures showed that genetic differences increase with increasing geographic distance.

Deep ocean channels appear to hinder dispersal to a greater extent than corresponding geographic distances over shallower continental shelf areas. The relationships between P or D and geographic distance (Fig. 4 and 6, respectively) revealed that in each case the largest residuals were from the comparison of Banquereau and St. Pierre banks. These two populations are relatively close together spatially (300 km) but widely separated genetically relative to the other comparisons. The two populations are separated by the deep Laurentian Channel (Fig. 1). We devised a "channel factor" (CF) to account for the relative dimensions of the two channels in our sampling area, the Fundian Channel and the Laurentian Channel: width at 360-m isobath $\times$ maximum depth. The choice of the 360-m isobath to establish width in the CF derives from observations that haddock are rarely captured below this depth. Haddock become obligate benthic feeders within their first year of life and are therefore closely associated with the sea bottom. An analysis of haddock catches by Canadian research trawlers from 1970 to 1990 (see Halliday and Koeller 1981 for a description of survey protocols) shows that only about 3% of the total catch in numbers is taken at depths greater than 180 m (100 fathoms). Seasonal differences were observed with 2.6% of the catch occurring at these depths in summer (see also Scott 1982), 2.9% in fall, and 3.7% in winter. When CF was included in regression analyses, it explained variation in D more effectively than geographic distance. This indication of the importance of the Fundian and Laurentian channels as barriers to dispersal for haddock is consistent with previous views of their significance (Halliday and Pinhorn 1990).

As noted above, genotype group III (Fig. 3) exhibited a cline in frequency of occurrence, from a low of 7% in the Georges Bank sample to 25% in the St. Pierre Bank sample (Fig. 8). Genetic clines are typically attributed to one of two causes: spatial trends in selective pressures acting on genotypes or gene flow between previously isolated genetic groups. The possibility that the cline in group III frequencies is generated by selection acting on nonneutral haplotypes cannot be ruled out; however, apart from a variety of mitochondrial genotypes associated with genetic disease in humans (Poulton 1988), there is no evidence in the literature of nonneutral mitochondrial genotypes in nature. We therefore favour the latter possibility, namely restricted gene flow mixing previously isolated haplotypes. This point emphasizes the restricted nature of dispersal among populations of haddock, since haddock were excluded from much of their present range by the late Wisconsin ice sheets (Mayewski et al. 1981); it implies that the approximately 8000–10 000 years that is the maximum age for modern haddock populations has been insufficient to homogenize the frequency of genotype group III over the 1000-km range sampled.

Since genotype group III shows a clinal distribution, it could be concluded that the correlation between D and geographic distance results solely from the cline. However, removal of genotype group III and recalculation of the genetic distances

based on the remaining data continues to show a significant correlation (based on the Monte Carlo randomization) between divergence (D) and distance ($r = 0.61$, $P \approx 0.05$). Hence, the genetic differentiation implied by the geographic trend in D is not solely a product of the cline in genotype group III.

Our results offer evidence that the Laurentian Channel and possibly the much smaller Fundian Channel function as significant barriers to gene flow between haddock spawning aggregations. Although we did not detect any significant indications of population divisions among haddock sampled on the Scotian Shelf, the evidence of restricted genetic interchange on broader geographic scales suggests that the current management policy of recognizing at least two stocks (western and eastern) on the Scotian Shelf is a wise one. The possibility that haddock home with high fidelity to their natal banks to spawn, and hence that even adjacent banks harbour distinct populations, remains viable. A clearer resolution of haddock population structure, as well as the phylogenetic questions raised above, will require considerable additional sampling effort. The economic importance of haddock as well as the intriguing nature of the biological questions involved suggests that such an effort would be well worthwhile.

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Genetic Population Structure of American Plaice (*Hippoglossoides platessoides*) from the Gulf of St. Lawrence, Canada

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The genetic population structure of American plaice (*Hippoglossoides platessoides*) was examined with an electrophoretic analysis of the products of 40 protein-coding loci and with restriction fragment length polymorphisms in mitochondrial DNA. Samples of American plaice collected in the same year from six sites within the Gulf of St. Lawrence showed little heterogeneity in allele frequencies at eight loci. Furthermore, few significant differences in allele frequencies were detected among American plaice collected over three different years from the same site. Also, fish collected from the Grand Banks of Newfoundland showed little genetic differentiation from the Gulf fish. Of the 25 restriction endonucleases used to search for restriction fragment length polymorphisms, only *Hind* III and *Pvu* II gave variable fragment patterns. Most fish had the same haplotype and three other haplotypes were represented by two fish at most from each site. American plaice in the Gulf of St. Lawrence appear to be a single, randomly mating population. Large effective population size and high rates of gene flow may account for the observed lack of genetic population subdivision.

Nous avons étudié la structure génétique des populations de plie canadienne (*Hippoglossoides platessoides*) d'après des analyses électrophorétiques des produits de 40 loci codants (des protéines) et en fonction du polymorphisme des sites de restriction de l'ADN mitochondrial. Dans des échantillons provenant de plies canadiennes capturées la même année à six endroits dans le golfe du Saint-Laurent, la fréquence des allèles de huit loci ne présentait qu'une faible hétérogénéité. De plus, on n'a enregistré que peu de différences importantes dans la fréquence des allèles chez des plies canadiennes prises au même endroit sur trois années. En outre, les spécimens capturés sur les Grands Bancs de Terre-Neuve présentaient peu de différenciation génétique par rapport à ceux qui provenaient du golfe. Des 25 endonucléases de restriction utilisées dans la recherche du polymorphisme des sites de restriction, seuls les *Hind* III et *Pvu* II présentaient des structures de fragments variables. La plupart des échantillons possédaient le même haplotype, et trois autres haplotypes étaient présents chez deux poissons tout au plus à chaque endroit. La plie canadienne du golfe du Saint-Laurent semble appartenir à une seule population où l'accouplement se fait de façon aléatoire. La taille réelle de la population, qui est élevée, et le fort taux de flux génétique pourraient être des facteurs qui expliquent le manque apparent de subdivision génétique dans la population.

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Stock delineation is a problem frequently encountered in fisheries management. Stock assessment, toward the goal of an optimal sustainable yield, can only be achieved with reliable knowledge of the genetic population structure of a species (Larkin 1981).

American plaice (*Hippoglossoides platessoides*) are an abundant Pleuronectiform fish distributed along the east coast of the northern United States, Canada, and the coasts of Greenland and Iceland (Scott and Scott 1988). They are managed as one stock in the southern Gulf of St. Lawrence (NAFO, North Atlantic Fisheries Organization, unit 4T) (Pitt 1982). However, tagging data suggest that two discrete stocks exist in the Magdalen Shallows in the southern Gulf of St. Lawrence (Powles 1965). One stock is thought to occur in the area from George's

Bay to St. Paul Island off the tip of Cape Breton and another in the Shippegan Gully to Orphan Bank region off the New Brunswick coast. These stocks are thought to be maintained by returns of adult fish to the same spawning areas each summer. More recently, it has been suggested that length-at-age differences observed between American plaice caught in these two areas could indicate the presence of two stocks in the southern Gulf (Tallman and Forest-Gallant 1990). There has been an intensive research effort in monitoring population dynamics and the landed values of fish in recent years, but the genetic population structure of American plaice is unknown. Information concerning genetic population structure is essential for the management and rehabilitation of this resource.

The assessment of genetic population structure requires analysis of defined genetic systems. Allozyme variants and restriction fragment length polymorphisms (RFLPs) in mitochondrial DNA (mtDNA) have become invaluable sources of genetic markers for stock delineation (Allendorf and Utter 1979; Ferris

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and Berg 1987). However, there are instances in which a single approach would have failed to identify genetically discrete stocks (Ferguson et al. 1991). As well, there are cases where the use of a single method fails to reveal the variation necessary for a genetic study of population structure. Studies using both methods may increase the likelihood of detecting gene flow between partially reproductively isolated units (Gyllensten and Wilson 1987) and may help detect differential gene flow between sexes. Therefore, using a variety of approaches is the most powerful method for detecting genetic population structure.

In this study, we examined the genetic population structure of American plaice in the Gulf of St. Lawrence with variation at 40 protein-coding loci and RFLPs in mtDNA. Sampling was repeated over three years so that changes in allele frequency over time could be examined. We also analyzed fish from the Grand Banks of Newfoundland so that the patterns observed within the Gulf could be put into a broader geographical perspective.

Materials and Methods

Sample Collection

Eye, liver, and white muscle were collected from 344 fish in NAFO region 4T in August 1988 in conjunction with the Department of Fisheries and Oceans Groundfish Survey (cruise H192) (Fig. 1). An additional 465 fish were collected from NAFO regions 4S, 4R, and 4T (cruises H203 and H204) in August 1989, and another 285 fish in August 1990 (cruises N141 and H219). Forty-three fish were collected from the Grand Banks of Newfoundland (43°51'N, 49°02'W) in May 1991 (NAFO cruise WT106). Juvenile and adult American plaice of

both sexes and ranging between 4 and 49 cm in total length were collected. All fish were collected using a Western IIA Otter trawl with 0.5-h tows, except for samples from cruise N141. On this cruise, fish were taken after 20-min tows with a URY 81/114' trawl.

In the area south of the Laurentian Channel, sample sites (Fig. 1) were chosen to reflect the location of the possible stocks suggested by Powles (1965). Throughout the rest of the Gulf, boundaries were chosen to represent possible natural barriers to gene flow such as the Laurentian Channel, Anticosti Island, and the distance across the Gulf of St. Lawrence.

Genetic Analysis

The protein products of 39 loci were resolved on cellulose acetate gels (Hebert and Beaton 1989), while an additional system (ACP) was resolved on a 12% starch gel (Allendorf et al. 1977) (Table 1). An initial screen with 25 restriction endonucleases was performed on mtDNA extracted from fresh and frozen egg samples to identify the subset of restriction enzymes that detected polymorphism. Egg samples collected during the 1990 sampling season were frozen in TEK buffer (Chapman and Powers 1984) with the addition of 0.25 M sucrose and stored at -20°C until analysis. Partially purified mtDNA (Chapman and Powers 1984) was digested with restriction endonucleases and loaded into 0.8% agarose gels; two lambda DNA standards, one digested with *Hind* III, and one digested with both *Hind* III and *Eco* RI, were run along side. The gels were stained with ethidium bromide, and the mtDNA fragments were viewed with a UV transilluminator.

A more detailed analysis was made with those restriction enzymes that produced variable fragment patterns. Total DNA extracts were obtained from liver samples from the 1989 col-

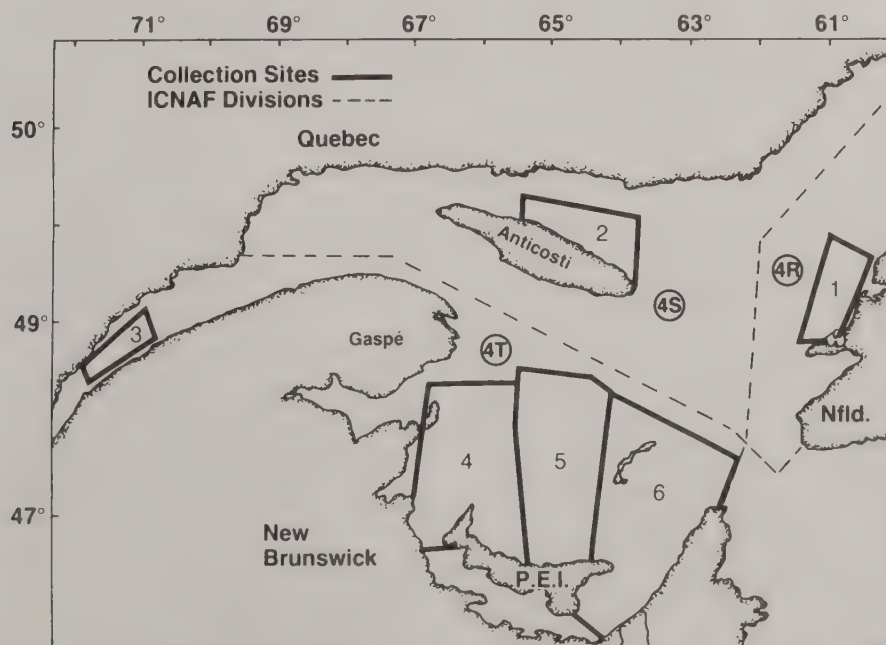


FIG. 1. Collection sites for American plaice in the Gulf of St. Lawrence, Canada. 1, Esquiman Channel; 2, North Anticosti Island; 3, St. Lawrence Estuary; 4, Shippegan Gully/Orphan Bank; 5, Central Gulf/Bradelle Bank; 6, George Bay/St. Paul Island.

TABLE 1. Proteins investigated in American plaice from the Grand Banks and Gulf of St. Lawrence. Enzyme commission numbers, buffer used, and the number of loci found are also given.

Protein (abbreviation)	EC No.	Tissue ^a	Buffer ^b	No. of loci
Acid phosphatase (ACP)	3.1.3.2	L	AC	1
Aconitase (ACO)	4.2.1.3	L	AC	1
Adenosine deaminase (ADA)	3.5.4.4	M	TG	2
Alcohol dehydrogenase (ADH)	1.1.1.1	L	TG	2
Creatine kinase (CK)	2.7.3.2	M/E	TG	3
Diaphorase (DIA)	1.6.4.3	L	TG	2
Fructose bisphosphate (FBP)	3.1.3.11	L	AC	1
Glucose-6-phosphate dehydrogenase (G6PDH)	1.1.1.49	M/E	AC	1
Glucosephosphate isomerase (GPI)	5.3.1.9	M	TG	2
Beta-glucuronidase (GUS)	3.2.1.31	L	TG	2
Glutamate dehydrogenase (GLUDH)	1.4.1.-	L	AC	1
Glycerate dehydrogenase (GLYDH)	1.1.1.29	L	AC	1
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	1.2.1.12	M	TC	2
Glycerol-3-phosphate dehydrogenase (G3PDH)	1.1.1.8	L	TG	2
Isocitrate dehydrogenase (IDHP)	1.1.1.42	M/L	AC	2
Lactate dehydrogenase (LDH)	1.1.1.27	M/E	AC	3
Malate dehydrogenase (MDH)	1.1.1.37	M	AC	2
Malic enzyme (MEP)	1.1.1.40	M	AC	2
Mannose-6-phosphate isomerase (MPI)	5.3.1.8	L	TG	1
Peptidases Glycyl-L-leucine (PEPA) and DL-Leucylglycylglycine (PEPB)	3.4.-.-	L	TG	2
Phosphoglucomutase (PGM)	5.4.2.2	M	TG	1
Purine nucleoside phosphorylase (PNP)	2.4.2.1	E	AC	1
Sorbitol dehydrogenase (SDH)	1.1.1.14	L	TG	1
Superoxide dismutase (SOD)	1.15.1.1	L	TG	1
Xanthine dehydrogenase (XDH)	1.1.1.20	L	TG	1

^aL = liver, M = muscle, E = eye.

^bAC = amine-citrate (pH 7.2) (Hebert and Beaton 1989), TG = Tris-glycine (pH 8.5), TC = Tris-citrate (pH 7.0) (Selander and Yang 1969).

lections (Saghai-Marooof et al. 1984). After digestion with a restriction endonuclease and electrophoretic separation as described above, the mtDNA fragments were transferred onto nylon membranes (Hybond-N, Amersham). The membranes were probed with purified American plaice mtDNA, prepared from ultracentrifugation in cesium chloride of previously frozen liver and egg samples (after Lansman et al. 1981). Twenty-five nanograms of pure mtDNA was labelled with [α -³²P]dCTP and [α -³²P]dATP by random priming (Boehringer Mannheim) and separated from any unincorporated nucleotides using Sephadex G-50 columns (Maniatis et al. 1982). Hybridization and autoradiography were performed as described by Maniatis et al. (1982).

Data Analysis

Deviations from random genetic proportions at the protein-coding loci were tested using chi-square goodness of fit. Alleles occurring at a frequency of less than 0.05 were grouped together as a single allelic class for the analysis. Departures from random proportions may be an indication of selection or nonrandom mating.

Gene frequencies at polymorphic loci were calculated for each sex and sexes combined for each sample. A contingency chi-square analysis was used to test for allele count differences (1) between males and females within a site, within a year, (2) among collection sites (the Grand Banks fish were compared with the 1990 samples from the Gulf of St. Lawrence), and (3) between fish collected from the same site in different years. Allelic categories with less than five were grouped with the next largest class. Each set of tests was done on a single locus and then summed over all loci to provide an overall statistic.

Genetic distance (Nei 1975) was used to estimate the average proportion of nucleotide substitutions among samples. The proportion of variation accounted for by a particular level of population subdivision was calculated (Wright 1978), and the parameters (F_{IT} , F_{IS} , and F_{ST}) estimated components of variance of allele frequency (Weir and Cockerham 1984). A chi-square test (Waples 1987) was used to test the null hypothesis that $F_{ST} = 0$ for single locus F_{ST} values.

Variability was measured by calculating average expected heterozygosity and the effective number of alleles per locus (Kimura and Crow 1964). The effective number of alleles per locus may be less than the actual number of alleles, due to the presence of rare alleles that do not contribute to the overall level of heterozygosity. Thus, they are given less weight when the effective number of alleles is calculated. When the unweighted average is greater than the effective number of alleles, large effective population size (N_e) and high rates of gene flow are indicated, as these two characteristics result in the maintenance of rare alleles.

The chi-square analysis described by Roff and Bentzen (1989) was used to test for differences in the distributions of mtDNA clonal lines among the sites sampled in 1989.

Results

Protein-Coding Loci

The products of 35 loci were resolved in the samples collected in 1988 and polymorphisms were detected at four of these: phosphoglucomutase (PGM), glucose-6-phosphate isomerase (GPI), alcohol dehydrogenase (ADH), and adenosine deaminase (ADA) (Table 2). Five additional proteins were

TABLE 2. Allele frequencies in American plaice samples collected from the Gulf of St. Lawrence in 1988, 1989, 1990 and the Grand Banks in 1991. An additional 32 loci were monomorphic. Sampling locations (site codes in Fig. 1): GR, Grand Banks; SL, St. Lawrence Estuary (3); GB, George Bay/St. Paul Island (6); CG, Central Gulf/Bradelle Bank (5); SG, Shippegan Gully/Orphan Bank (4); EC, Esquiman Channel (1); AI, North Anticosti Island (2).

Locus	Allele	GR-91	SL-90	GB-88	GB-89	GB-90	CG-88	CG-89	CG-90	SG-88	SG-89	SG-90	EC-89	EC-90	AI-89	AI-90
ADA-2	124	0	0	0	0.014	0	0	0.007	0	0	0.013	0.009	0	0	0	0.010
	114	0.023	0.131	0.047	0.103	0.100	0.016	0.098	0.030	0.008	0.086	0.083	0.092	0.111	0.050	0.094
	100	0.953	0.857	0.836	0.863	0.878	0.881	0.874	0.931	0.956	0.884	0.880	0.875	0.867	0.936	0.833
	79	0.012	0.012	0.108	0.020	0.022	0.088	0.021	0.039	0.036	0.017	0.028	0.033	0.022	0.014	0.042
	57	0.012	0	0.009	0	0	0.015	0	0	0	0	0	0	0	0	0.021
ADH-1	25	0.047	0.012	0.041	0.021	0.033	0.060	0.028	0.029	0.043	0.009	0.037	0.017	0.067	0.014	0
	-100	0.953	0.988	0.959	0.979	0.967	0.940	0.972	0.971	0.957	0.991	0.963	0.983	0.933	0.986	1.00
GAPDH	100	0.628	0.471	—	0.562	0.500	—	0.531	0.549	—	0.478	0.481	0.433	0.489	0.514	0.500
	78	0.372	0.529	—	0.438	0.500	—	0.469	0.451	—	0.522	0.519	0.567	0.511	0.486	0.500
GPI-1	167	0.012	0.012	0	0.014	0.011	0.010	0.007	0.068	0.020	0.004	0.046	0.017	0.022	0.007	0.021
	133	0.464	0.488	0.405	0.411	0.489	0.374	0.441	0.520	0.377	0.448	0.454	0.308	0.467	0.413	0.448
	100	0.477	0.452	0.519	0.534	0.467	0.515	0.521	0.373	0.525	0.522	0.500	0.558	0.433	0.507	0.469
	78	0.035	0.012	0.067	0	0.022	0.096	0.031	0.039	0.066	0.026	0	0.109	0.078	0.073	0.052
	30	0.012	0.036	0.009	0.041	0.011	0.005	0	0	0.012	0	0	0.008	0	0	0.010
MPI	110	0	0.024	—	0.021	0.033	—	0.046	0	—	0.013	0.009	0	0.022	0	0.010
	100	0.884	0.821	—	0.785	0.811	—	0.797	0.853	—	0.828	0.787	0.833	0.789	0.870	0.771
	89	0.116	0.155	—	0.194	0.156	—	0.157	0.147	—	0.159	0.204	0.167	0.189	0.130	0.219
PGM	150	0	0.012	0	0.007	0	0	0	0.020	0	0	0.019	0.008	0	0	0
	117	0.256	0.119	0.145	0.108	0.133	0.122	0.143	0.196	0.131	0.171	0.120	0.117	0.167	0.080	0.104
	100	0.732	0.845	0.814	0.865	0.789	0.812	0.815	0.775	0.813	0.803	0.842	0.817	0.800	0.855	0.865
	80	0.012	0.024	0.041	0.020	0.078	0.061	0.042	0.029	0.056	0.026	0.019	0.050	0.033	0.065	0.031
	55	0	0	0	0	0	0.005	0	0	0	0	0	0.008	0	0	0
SDH	340	0.035	0.024	—	0.035	0.067	—	0.047	0.030	—	0.078	0.028	0.050	0.033	0.043	0.052
	260	0.186	0.179	—	0.236	0.144	—	0.173	0.137	—	0.157	0.139	0.158	0.156	0.243	0.167
	200	0.012	0.035	—	0.049	0.067	—	0.057	0.059	—	0.030	0.111	0.092	0.089	0.064	0.010
	100	0.721	0.583	—	0.479	0.422	—	0.424	0.490	—	0.426	0.481	0.492	0.489	0.429	0.511
	0	0.046	0.179	—	0.201	0.300	—	0.299	0.284	—	0.309	0.241	0.208	0.233	0.221	0.260
SOD	400	0	0	—	0.007	0.011	—	0	0.010	—	0	0	0.017	0	0	0
	100	0.965	0.988	—	0.979	0.967	—	0.979	0.980	—	0.974	0.981	0.983	0.978	0.972	0.990
	-150	0.035	0.012	—	0.014	0.022	—	0.021	0.010	—	0.026	0.019	0	0.022	0.028	0.010
H _e ^a		0.051	0.063	—	0.064	0.067	—	0.067	0.064	—	0.065	0.070	0.067	0.071	0.061	0.065
H _e ^b		—	—	0.032	0.034	0.033	0.035	0.031	0.030	0.038	0.040	0.030	0.032	0.033	0.027	0.031
N		43	42	113	73	45	103	143	51	128	115	54	60	45	69	48

^aBased on 40 loci.

^bBased on 35 loci.

resolved in the 1989 and 1990 samples as well as fish from the Grand Banks, and of these, four were polymorphic: sorbitol dehydrogenase (SDH), mannose-6-phosphate isomerase (MPI), superoxide dismutase (SOD), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Three of 81 tests detected significant differences in allele frequencies between males and females from the same sample (George's Bay/St. Paul Island (GB)-88, *PGM**; Esquiman Channel (EC)-89, *GAPDH-I**; North Anticosti Island (AI)-90, *GAPDH-I**); this is fewer than expected by chance alone. No overall difference was found between the sexes for any sample when all loci were combined. Therefore, data for males and females were combined for the other chi-square tests.

Few significant deviations from random mating expectations were found when the chi-square goodness of fit statistic was used to test for departures from genotypic proportions. Despite the 81 tests performed, only five significant deviations were detected (Shippegan Gully/Orphan Bank (SG)-89, *GAPDH-I**; Central Gulf/Bradelle Bank (CG)-89, *GAPDH-I**; AI-90, *GPI-I**; EC-90, *SDH**; St. Lawrence Estuary (SL)-90, *GAPDH-I**). No deviations from random mating expectations

were found for any sample within any year when all loci were combined.

Contingency chi-square analysis showed little consistent heterogeneity in allele frequencies among sample sites within years; 3 of 20 tests were significant. American plaice collected from three sites in 1988 differed in frequency at *ADA-2**, while those collected from five sites in 1989 differed at *GPI-I**. The American plaice from the Grand Banks (GR) and those collected from the Gulf of St. Lawrence in 1990 differed at *SDH**.

Allele frequencies of samples collected from the same site in different years also showed few differences; 4 of 39 tests were significant. Significant heterogeneity was detected at *GPI-I** among fish sampled over three years from both CG and GB. A comparison of the sites sampled over two years revealed significant changes in only two cases (EC, *ADA-2**, CG, *GPI-I**).

The values of Nei's genetic distance were small ($<<0.0001$) and indicated very little nucleotide substitution among sampling sites. This was also supported by the gene diversity analysis (Table 3). Values of F_{ST} were not significantly different from zero; the only locus with a significant F_{ST} value was *ADA-2**.

TABLE 3. Effective versus average number of alleles and F statistics for American plaice from the Gulf of St. Lawrence. Four loci were used to calculate F statistics in 1988 samples and six loci in 1989 and 1990 samples; most common alleles had frequencies <95% for these loci.

Measure	1988	1989	1990
Number of alleles			
Effective	1.062	1.143	1.140
Average	1.314	1.525	1.525
F statistics ^a			
F_{ST}	0.0042 $\pm$ 0.0048	0.1051 $\pm$ 0.3629	-0.0255 $\pm$ 0.0499
F_{IT}	0.0568 $\pm$ 0.0233	0.0871 $\pm$ 0.0118	0.0396 $\pm$ 0.1280
F_{IS}	0.0531 $\pm$ 0.0232	0.0905 $\pm$ 0.0931	0.0574 $\pm$ 0.1538

^a F_{IS} = diversity among individuals in a sample area; F_{ST} = diversity among sample areas; F_{IT} = overall inbreeding coefficient.

TABLE 4. Fragment sizes produced by restriction endonuclease digestion of mtDNA isolated from American plaice from the Gulf of St. Lawrence.

Endonuclease	Pattern	Fragment sizes (kilobase pairs)
<i>Apa</i> I	A	11.76, 2.58, 2.26, 1.36
<i>Ava</i> I	A	8.85, 7.41
<i>Ban</i> I	A	7.79, 5.20, 2.59, 2.16
<i>Ban</i> II	A	4.30, 2.48, 1.74, 1.33, 1.24
<i>Cla</i> I	A	5.70, 4.21, 3.47, 1.72
<i>Dra</i> I	A	8.66, 3.61, 3.16
<i>Dra</i> II	A	4.53, 3.65, 2.18, 1.97
<i>Hae</i> II	A	8.30, 6.19, 5.74
<i>Hinc</i> II	A	3.38, 2.57, 2.43, 2.14, 1.90
<i>Hind</i> III	A	3.84, 3.67, 3.19
	B	3.67, 3.19, 2.12
<i>Hpa</i> I	A	14.48, 4.47
<i>Mam</i> I	A	8.71, 4.90
<i>Nco</i> I	A	15.67, 2.90
<i>Pst</i> I	A	8.95, 5.19
<i>Pvu</i> II	A	6.17, 3.86, 3.52, 3.04
	B	7.39, 6.17, 3.52
<i>Sst</i> I	A	2.92, 2.71, 2.51, 1.96, 1.24, 1.02
<i>Xba</i> I	A	7.67, 6.62, 4.98

The estimate of F_{ST} for the 1990 samples had a negative value. Even though the definitions in Weir and Cockerham (1984) do not allow negative values, the corrections used for the finite sample sizes may result in the F statistics being less than zero (Waples 1987). The F statistics indicate a slight deficiency in heterozygotes compared with Castle-Hardy-Weinberg expectations, but this is not consistent over time or within sampling areas. Furthermore, most of the variation occurs within sampling areas.

Little variation in heterozygosity occurs among sites or among years. The values of average expected heterozygosities (H_e) in 1988 are somewhat lower than those calculated for the other two sampling years (Table 2). The estimate for 1988 is based on data from 35 loci, while the 1989 and 1990 estimates are based on data from 40 loci. Four of the five additional loci used in the 1988 and 1989 calculations were variable resulting in the higher estimates. When the subset of 35 loci common to all three years is used to estimate heterozygosity (Table 2), this difference disappears.

Mitochondrial DNA

Of the 25 restriction endonucleases used to search for mtDNA polymorphism in American plaice, 17 cut the molecule more than once (Table 4), and eight (*Bam* HI, *Bgl* II, *Csp*45 I, *Eae* I, *Eco* RI, *Eco* RV, *Sal* I, and *Sca* I) cut the molecule once or not

TABLE 5. Numbers of American plaice from the Gulf of St. Lawrence with specific restriction fragment patterns. Key to sample sites is given in Table 2 and pattern designations in Table 4.

Pattern		Sample site				
<i>Pvu</i> II	<i>Hind</i> III	EC	AI	SG	CG	GB
A	A	6	4	8	7	7
A	B	1	0	0	0	0
B	A	2	1	1	1	1
B	B	0	0	0	1	0

at all. Only *Hind* III and *Pvu* II produced variable fragment patterns. The nucleon diversity (Nei and Tajima 1981) was low ($h = 0.0170$) and 32 of 40 fish had the most common haplotype (Table 5). Three additional haplotypes were represented by two fish at most in each of the sampling sites. The chi-square analysis detected no significant differences in haplotype frequencies among fish captured from the sites surveyed in 1989.

The average length of the mtDNA molecule in American plaice was estimated to be 15 299 $\pm$ 3090 base pairs. This estimate is on the low end of the range reported for animals (15 700 to 19 500 base pairs) (Brown 1983) but might be attributed to the methods used. Neither ethidium bromide staining nor southern blot hybridization allows the detection of small fragments (Gyllenstein and Wilson 1987). This does not affect the results because differences in fragment patterns rather than molecule size were used to examine population structure.

Discussion

Genetic Population Structure

American plaice in the Gulf of St. Lawrence show no evidence of population subdivision. Fish from different sites show no consistent differences in allele frequencies at enzyme-coding loci, and samples have calculated values of Nei's genetic distance well below that expected for subdivided species (Avice 1974). Gene diversity analysis also provides no reason to reject the null hypothesis that American plaice in the Gulf of St. Lawrence exist as a single genetic population. The low levels of inbreeding indicate little deviation from random mating. Moreover, no differences in mtDNA haplotype frequencies were found among fish from different sample sites. These results do not support the hypothesis of multiple stocks of American plaice in the Gulf of St. Lawrence as was suggested by Powles (1965). Interestingly, inclusion of the Grand Banks fish does not alter this conclusion.

Other flatfish species also show little population subdivision. Small genetic distances occur among populations of Pacific

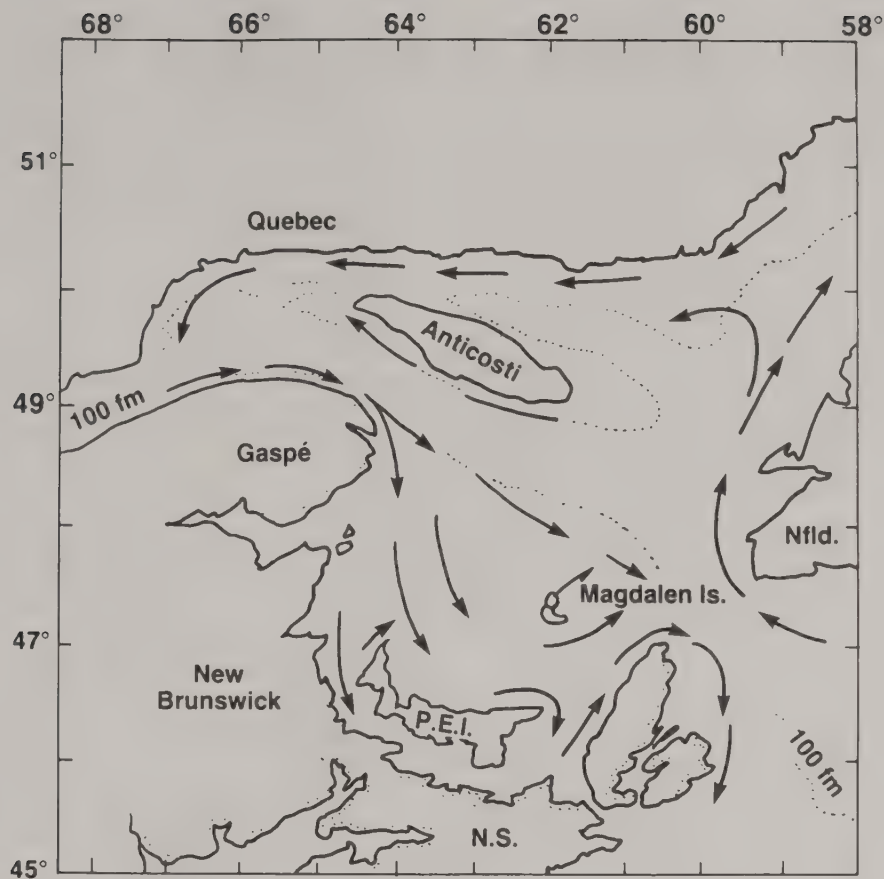


FIG. 2. Spring and early summer surface currents in the Gulf of St. Lawrence, Canada (from Bumpus and Lauzier 1965).

halibut (*Hippoglossus stenolepis*) (Grant et al. 1984), plaice (*Pleuronectes platessa*) from estuaries along the north west coast of Brittany (Brulé 1989), and *P. platessa* sampled from different parts of the North Sea (Purdom et al. 1976; Ward and Beardmore 1977). In contrast, Fairbairn (1981a) found some genetic differences among Greenland halibut (*Reinhardtius hippoglossoides*) from the Northwest Atlantic and the Gulf of St. Lawrence but no differences within the Gulf of St. Lawrence. A recent subdivision among yellow sole (*Buglossidium luteum*) from the Mediterranean coasts of Algeria and France was suggested by Alili and Berrebi (1989). Six genetically distinguishable populations of witch flounder (*Glyptocephalus cynoglossus*) were found around the coasts of Newfoundland (Fairbairn 1981b).

The effective number of alleles per locus compared with the average number of alleles per locus suggests that the effective population size of the American plaice in the Gulf of St. Lawrence is large. The effective number of alleles is lower than the average number of alleles in all three years. The presence of rare alleles accounts for this difference. Large effective population sizes will maintain rare alleles in the absence of any strong selective forces. Even when gene flow is limited, a large effective population size will prevent differentiation of allele frequency through genetic drift (Kimura and Crow 1964). A similar difference between the average and effective number of alleles in yellowtail flounder (*Pleuronectes ferrugineus*,

previously *Limanda ferruginea*) was suggested as an indicator of a large effective population size in that species (McCafferty 1982).

The amount of mtDNA haplotypic variation within and between localities in American plaice is small. Similar results have also been reported for some other marine species of fish (review: Ovenden 1990). For example, in the orange roughy (*Hoplostethus atlanticus*), 1 of 11 haplotypes was predominant in fish from both the east and west coasts of Tasmania (Ovenden et al. 1989). As in American plaice, the remaining haplotypes occurred at very low frequencies in all sampling areas. A slower rate of mitochondrial genome evolution in marine species, recent bottleneck events, or high levels of family-specific mortality are possible explanations for the low levels of diversity observed (Ovenden 1990). In those marine species where population subdivision has been detected (e.g. toadfish and catfish – Avise et al. 1987), the potential for dispersal is much reduced due to the lack of a larval phase in their life history (Ovenden 1990).

The mtDNA analysis provided less resolution of population structure than did the allozyme analysis because of lower inherent variability. A similar result was observed for brook trout (*Salvelinus fontinalis*) from Cape Race, Newfoundland (Ferguson et al. 1991). Reductions in the population sizes during recent historical events could explain this incongruence. Bottlenecks are expected to have greater effects on mtDNA

variability than nuclear DNA variability because the effective population size of mtDNA is much lower than that of nuclear DNA (Ferris and Berg 1987). Thus, the survivors of bottlenecks will be relatively homogeneous for few mtDNA types but will have retained greater nuclear DNA variation, a pattern consistent with the data. Even with high gene flow, lower than expected mtDNA haplotype variation may occur as a result of fluctuations in population size or as a result of overlapping generations in the life history (Avisé et al. 1988).

Knowledge of how fish are partitioned in time as well as in space is important for sound management decisions. If fluctuations in allele frequencies exist or if different amounts of population differentiation are found from one year to the next, it becomes difficult to define stock boundaries. American plaice were sampled over a 3-yr period at about the same sampling sites and at the same time of year. The population structure did not vary significantly and so, our conclusions are not confounded by temporal change. In contrast, many studies of genetic population structure do not consider changes in allele frequency over time. In those that do, however, the results indicate the complexities and difficulties in delineating stocks. For instance, low amounts of genetic divergence were found among populations of fourhorn sculpin (*Myoxocephalus quadricornis*), but shifts in allele frequencies over time suggested that populations were not geographically stable (Gyllenstein and Ryman 1985).

Mechanisms Maintaining Population Structure

High rates of gene flow among American plaice could maintain the observed population structure. Dispersal from spawning grounds during the embryonic period may be important in this regard. American plaice make annual migrations to shallow areas to spawn (Powles 1965). Some combination of bottom type, temperature, and depth determines where and when spawning occurs. As a result, spawning can occur over a wide area depending on the conditions during the spawning season (Pitt 1966). In the Gulf of St. Lawrence, American plaice spawn from mid-April to mid-May when both mature and immature fish are found inshore at depths of 40–200 m. Areas with highest concentrations of fish are Chaleur Bay, the Shippegan Gully (northwest of Prince Edward Island), and the northwest tip of Cape Breton (Powles 1965). Little information exists on the distribution and habits of American plaice in the north of the Laurentian Channel. They are found in greatest numbers in the Esquimaux Channel, off the north east shore of Anticosti Island, near Sept-Îles, and along the North Shore between Pointe de Natashquan and Cape Whittle (Ouellet and Tremblay 1990). Spawning may occur near these areas, in the late spring, as it does in the southern Gulf. A study of American plaice from the Scotian Shelf showed that given the time from spawning to hatching, 7 d at 10°C (Neilson et al. 1988), or 11–14 d at 4°C (Russell 1976), the eggs could not move more than 14 km. The potential for movement of American plaice during the same time period is greater in the Gulf of St. Lawrence. Given an average current speed of about 8 km/d (Bumpus and Lauzier 1965), American plaice could potentially move between 56 and 88 km before hatching and another 8–12 km during the free embryo phase. The motion of surface currents in the late spring and early summer (Fig. 2), along with a large and variable spawning ground, may facilitate widespread dispersal of American plaice.

Movements of adults may also contribute to the mixing of plaice in the Gulf of St. Lawrence. Mature fish move into deeper waters along the Laurentian Channel in the early fall (Powles

1965), and they may even cross the Channel as do Atlantic halibut (*Hippoglossus hippoglossus*) (Stobo et al. 1988). Tagging studies indicate that older individuals can move over large distances (Powles 1965). Therefore, there is a potential for an individual to move to and spawn in an area other than its natal grounds. North Sea Plaice are thought to share spawning grounds, and this is supported by few genetic differences among adult plaice from different areas (Brulé 1989). A similar situation may occur in the Gulf of St. Lawrence.

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Hooded Seal (*Cystophora cristata*) Pup Production in the Gulf of St. Lawrence

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Systematic visual aerial surveys were flown in the southern Gulf of St. Lawrence during March 1990 and 1991 to estimate hooded seal (*Cystophora cristata*) pup production. In 1990, the main whelping patch, occupying an area of 264 km², was located near Cape Breton Island. One hundred and five pups were counted on 17 transects, resulting in a mean density of 1.74 pups·km⁻² and an estimated pup production of 1638 (SE = 466). In 1991, a large whelping patch was located to the west of the Magdalen Islands, a second off the coast of Prince Edward Island, and a small patch was later found south of the Magdalen Islands. The two major patches were surveyed, resulting in a pup production estimate of 1564 (SE = 101). During the 1991 surveys, an estimated 71–93% of the pups were on the ice. Correcting for the distribution of births results in a minimum 1991 pup production estimate of 2006 (SE = 190) for the Gulf of St. Lawrence.

Des relevés visuels aériens systématiques ont été effectués dans le sud du golfe Saint-Laurent en mars 1990 et 1991 pour déterminer la production de jeunes phoques à capuchon (*Cystophora cristata*). En 1990, la zone principale de mise bas, couvrant un territoire de 264 km², a été localisée près de l'île du Cap Breton. Cent cinq petits ont été dénombrés au cours des 17 transects, soit une densité moyenne de 1,74 petits·km⁻² et une production de petits estimée à 1 638 (SE = 466). En 1991, une grande zone de mise bas a été localisée à l'ouest des Îles-de-la-Madeleine, une deuxième, au large de la côte nord de l'île-du-Prince-Edouard et une petite zone, localisée plus tard au sud des Îles-de-la-Madeleine. Les relevés ont été effectués dans les deux zones principales, indiquant une production de petits estimée à 1 564 (SE = 101). Au cours des relevés de 1991, on a estimé que 71–93% des petits étaient présents sur la banquise. Après ajustement pour tenir compte de la distribution des naissances, la production minimale de petits pour le golfe Saint-Laurent est estimée à 2 006 (SE = 190).

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The hooded seal (*Cystophora cristata*) is a large phocid inhabiting pelagic waters of the North Atlantic. Whelping occurs on the pack ice around Jan Mayen Island, in Davis Strait, and off eastern Canada. In the Northwest Atlantic, the majority of pups are born in Davis Strait and off the southeastern Labrador and northern Newfoundland coasts, an area known to sealers as the "Front" (Sergeant 1974). Aerial surveys flown in 1984 produced estimates of 19 000 (95% CI = 14 000–23 000) pups in Davis Strait and 62 000 (95% CI = 43 700–89 400) pups on the Front (Bowen et al. 1987b).

Hooded seals also whelp in the Gulf of St. Lawrence, but little is known of Gulf pup production or of the relationship between Gulf and Front hooded seals. In 1894, a single ship reportedly took 7562 animals consisting largely of hooded seals (Mosdell 1923; Horwood 1977). Throughout the early 1900's the population apparently declined, and in 1972 the commercial hunt for hooded seals was closed in the Gulf to protect the stock (Reeves and Ling 1981). Visual estimates of pup production from the 1980's ranged from 500 to 800 animals (D. E. Sergeant and W. Hoek, Department of Fisheries and Oceans, Mont Joli, Que. G5H 3Z4, unpubl. data; Stenson and Wakeham 1986), but this population has never been systematically surveyed.

Hooded seals give birth during a 2-wk period in March. Lactation period is short, lasting only 4 d, during which the pups

double in size from 22 kg at birth to 43 kg at weaning (Bowen et al. 1985, 1987a). After weaning, the pups may enter the water and disperse. Any attempt to estimate pup production must therefore correct for pups not born at the time of the survey and for pups that have already left the ice (Bowen et al. 1987b).

Since protection in 1972, it is likely that the hooded seal population in the Gulf has increased. Information on hooded seal diet indicates that hooded seals may compete with fishermen by feeding on commercially important fish (Stenson et al. 1991). In order to assess the impact of hooded seals on commercial fisheries, information on population size is required. In this paper, we estimate hooded seal pup production in the Gulf of St. Lawrence using visual aerial surveys and a model to correct for pups not born at the time of the survey (Bowen et al. 1987b; Stenson and Myers 1988).

Methods and Materials

Whelping occurs on the pack ice some 30 miles (48 km) south of the Magdalen Islands near Prince Edward Island or off the Cape Breton Island coast in the southern Gulf of St. Lawrence (Sergeant 1974; D. E. Sergeant and W. Hoek, unpubl. data) (Fig. 1). In this area the direction of ice drift is to the northeast along the Cape Breton Island coast and out to the Atlantic Ocean through Cabot Strait (El-Sabbh 1976).

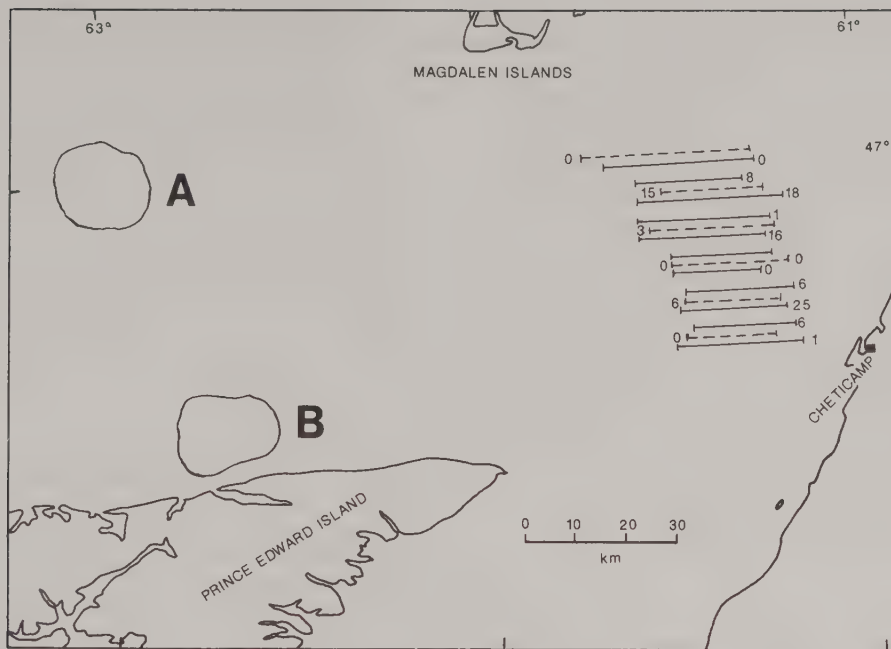


FIG. 1. Location of transects flown during the 1990 survey. Numbers of animals observed on each transect are plotted at the end of each transect line. Solid lines were flown 19 March 1990; broken lines were flown 20 March 1990. A and B represent the locations of the Magdalen Island and Prince Edward Island patches, respectively, surveyed in 1991.

Survey Design

Systematic strip transect surveys were flown in 1990 and 1991 in a Bell 206 helicopter equipped with Loran C for navigation. Altitude was maintained using a pressure altimeter calibrated with the recorded pressure at Cap aux Meules airport (1990) or with a radar altimeter (1991).

In 1990, whelping occurred in a single patch. Two surveys were flown. In the first survey, transects were evenly spaced at intervals of 3.6 km. During the second survey, transects were spaced 7.2 km apart, with the first transect offset from the previous day's lines by 1.8 km. Both surveys were flown at an altitude of 61 m. Observers were seated in the left front seat beside the pilot and right rear seat. Each observer counted seals in a strip 80 m wide on his side of the aircraft. Strips were delineated by aligning sighting marks on the aircraft window with distances measured on the ice with a metre wheel and marked with fluorescent powder.

The 1990 survey had a coefficient of variation of 28%. In 1991, we attempted to reduce this by increasing survey intensity. Two whelping patches, a Prince Edward Island patch and a Magdalen Island patch, were surveyed. In both surveys, evenly spaced transects 0.9 km apart were flown perpendicular to the long axis of the patch. The survey of the Prince Edward Island patch was flown at an altitude of 91 m and had a strip width of 200 m on each side of the aircraft. During the survey of the Magdalen Island patch, we discovered harp seal (*Phoca groenlandica*) beaters in the northern part of the hooded seal patch. In order to distinguish between solitary hooded seal pups and harp seal beaters, we reduced the altitude to 61 m and the strip width to 120 m on each side of the aircraft.

Data Analysis

Survey data were analysed using the methods outlined by Kingsley and Hammill (1991). The estimate for the number of

pups is given by $N_i = k_i (x_{i1}/2 + \sum_{j=2}^{J_i-1} x_{ij} + x_{iJ}/2)$ where

J_i = the number of transects in the i th group, x_{i1} is the number of pups counted on the first transect in the i th group, x_{ij} is the number of pups counted on the j th transect in the i th group, and k_i is a weighting factor equal to the transect spacing divided by the strip width of the i th group. The serial difference method for calculating error variance (Kingsley et al. 1985) was modified to take into account that the statistic of interest is the population total and not the spatial density of animals (Kingsley and Hammill 1991). Error variance for total numbers of seals in each group was then calculated using $V_i = k_i (k_i - 1)/2 \times \sum_{j=1}^{J_i-1} (x_j - x_{j+1})^2$ where the end transect is outside the patch.

The combined estimate for the total population was $N = \sum_{i=1}^I$

N_i , $V = \sum_{i=1}^I V_i$ where I is the number of groups of transects.

Stage Determination

In 1991, pups were classified into four identifiable stages based on morphology and presence or absence of the female (Bowen et al. 1987b; Stenson and Myers 1988). The two whelping patches were each surveyed three times. In each survey, transects spaced approximately 2 km apart were flown per-

pendicular to the long axis of the herd from one end of the patch to the other. Transects were flown at an altitude of 15 m, and all pups seen within 50 m were assigned a developmental stage. Information on the proportions of animals in each of these stages was used to model the distribution of births over time, which provided an estimate of the proportion of pups on the ice at the time of the survey. We used the model and computer programs developed for hooded seals by Bowen et al. (1987b). Confidence limits for the parameters of the birthing distribution were calculated by the log-likelihood for pairs of parameter values. The approximate 95% confidence region is the set of parameter values where the difference in the log-likelihood is approximately greater than 4 from the maximum (Cox and Hinkley 1974). Population estimates from the visual survey were corrected for pups not born at the time the survey was flown by dividing by the estimated proportion of pups present on the ice at the time of the survey. During our surveys and while working on the ice, no pups were observed in the water. Therefore, we fixed the proportion of stage 4 pups on the ice at 100%. The standard error of the corrected estimate was obtained from the standard formula for the variance of ratios of two random variables (equation 10.17; Stuart and Ord 1987).

Results

In 1990, a weaned hooded seal pup was observed in the harp seal patch, north of Magdalen Islands (47°N, 62°W), on 11 March, indicating that pupping had begun as early as 7 March. Reconnaissance flights flown 14 March, to the south and southeast of the Magdalen Islands, located hooded seals and pups near Cheticamp off Cape Breton Island over an area of 264 km² of ice (Fig. 1). No other hooded seal patch was located. Systematic strip-transect surveys of the hooded seal patch were flown on 19 and 20 March. During the first survey, 11 transects were flown. Survey intensity was 4.3% of a total survey area of 929 km². Eighty-one pups were counted on transect, resulting in an estimate of 1875 (SE = 556) animals. On 20 March, the patch was surveyed a second time. Six transects were flown between 46°41'N and 47°01'N, resulting in a survey intensity of 2.2% of a total area of 929 km². Twenty-four pups were counted on transect resulting in an estimate of 1111 (SE = 687) pups. Based on Loran C coordinates for limits of the herd, there appeared to be very little movement of the whelping patch between the two days of surveys. Combining the two surveys and treating them as one survey resulted in a mean density estimate of 1.74 animals·km⁻² and total pup production not corrected for the distribution of births over time of 1638 (SE = 466).

Mean counts were compared between the front and rear observers to test for any major differences. Mean number of pups counted per transect were 3.76 (SE = 1.20) and 2.41 (SE = 0.91) for the front and rear observers, respectively, but the differences were not significant ($t = 0.9$, $df = 32$, $p > 0.05$).

On 11 March 1991, whelping hooded seals (the Magdalen Island patch) were observed with some harp seals present at 47°38'N, 62°12'W. A second, very large patch (the Prince Edward Island patch) was observed on 14 March off Prince Edward Island (46°33'N, 62°43'W) (Fig. 1, 2). Hooded seals were also seen on 16 March at 46°42'N, 62°08'W, but we were unable to locate them until the final day when the helicopter was returning to Halifax.

The Prince Edward Island group distributed over 266 km² of ice was surveyed 18 March. A total of 412 pups was counted

on the 19 transects, resulting in an estimate of 954 (SE = 69) pups and a survey coefficient of variation of 7%. Survey intensity was 44%. The second patch (Magdalen Island patch), surveyed 19 March, covered an area of 158 km² and was located about 64 km south southwest of the Magdalen Islands (Fig. 2). One hundred and fifty-eight animals were counted on the 14 transects, resulting in an estimate of 610 (SE = 73) pups and a survey coefficient of variation of 12%. Survey intensity was 27%.

Little difference was observed between observers for the mean number of seals counted per line. In the Prince Edward Island patch, the left observer counted an average of 9.79 (SE = 2.03) pups per transect, and the right observer counted an average of 11.89 (SE = 2.64, $t = 0.6$, $df = 36$, $p > 0.05$) pups per line. In the Magdalen Island patch, the left observer's counts ($\bar{x} = 5.79$, SE = 1.72) were slightly higher than the right observer's counts ($\bar{x} = 5.50$, SE = 1.5, $t = 0.12$, $df = 26$, $p > 0.05$).

Surveys of the Prince Edward Island patch to determine the distribution of pup births began on 15 March. Five solitary (stage 4) pups were observed at this time, indicating that pupping began at least as early as 11 March. No new pups were observed in this patch after 22 March (K. Kovacs, pers. comm.). To calculate the distribution of births over time, we assumed that pupping started on 11 March and ended on 22 March and that 100% of the stage 4 pups were on the ice at the time of the survey. Using a beta distribution to fit the distribution of births over time, we estimated that 71% (SE = 0.06%) of the pups had been born at the time of the 18 March 1991 survey. Applying this correction to the survey data resulted in an estimate of 1350 pups (SE = 150) for the Prince Edward Island patch.

Staging surveys of the Magdalen Island patch were first flown on 11 March (Table 1). The presence of fat blueback pups (stage 3) indicated that pupping may have begun as early as 8 March. During the final staging survey of this patch on 19 March, some thin blueback (stage 2) pups, but no newborn (stage 1) pups, were observed. To calculate the distribution of births over time, we assumed that pupping began 9 March and was complete by 22 March. At the time of the 19 March 1991 survey, we estimated that 93% (SE = 0.02) of the births had occurred. Applying this correction to the survey data resulted in an estimate of 656 pups (SE = 80) for the Magdalen Island patch.

Combining these estimates, total hooded seal pup production in the Gulf of St. Lawrence was 2006 (SE = 190) in 1991.

Discussion

Visual aerial surveys may be subject to negative biases owing to difficulties in detecting all animals lying within the survey strip (Caughley 1974; Caughley et al. 1976). For Gulf hooded seals, we do not believe that this is a serious source of error in our estimates. The dark hooded seal pup is easily detected on the white ice. Also, the relatively low densities and the presence of the large female and often at least one male in the vicinity (Kovacs 1990) facilitate pup detection. Some pups hidden by ice ridges could have been missed, but the similarity in estimates obtained between years for surveys flown at similar times suggests that this is not a serious source of error or that it is at least consistent.

A more serious source of bias results from failure to detect all major patches of whelping animals (Myers and Bowen 1989). In 1990, a few hooded seal pups were observed dispersed

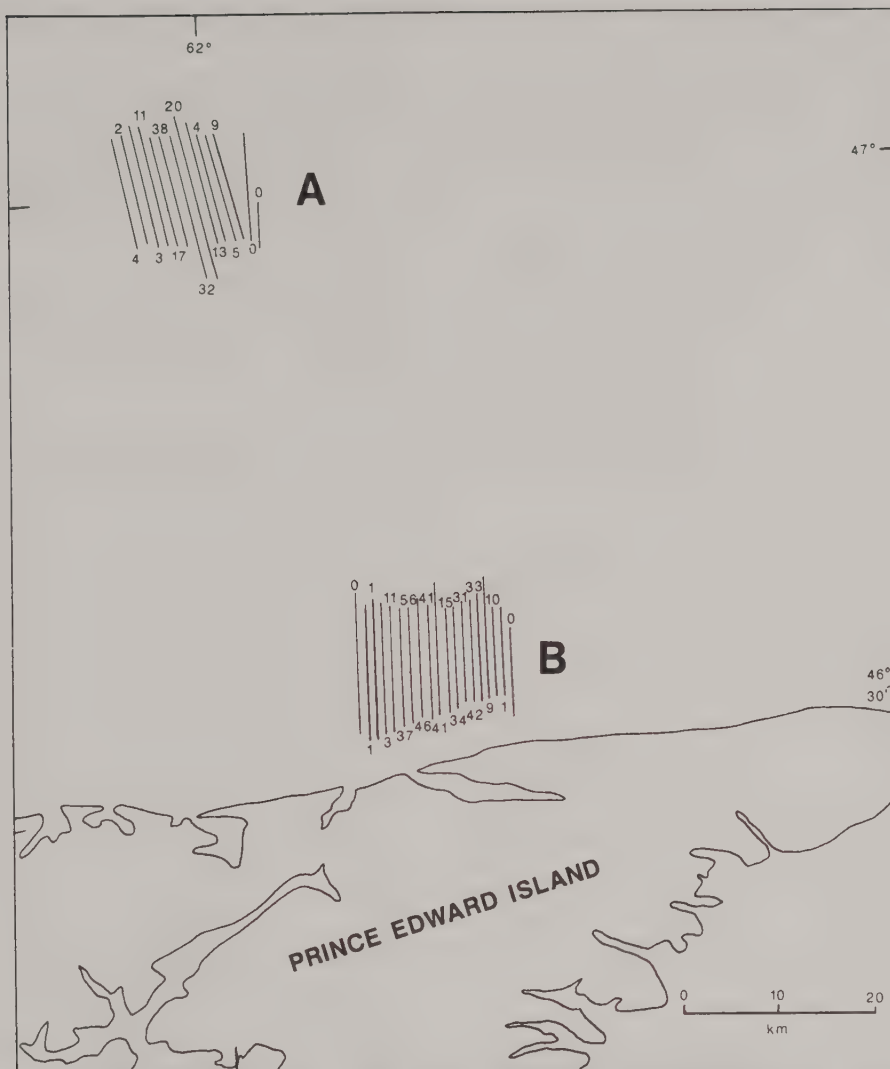


FIG. 2. Location of survey lines flown in 1991 surveys of the (A) Magdalen Island patch and (B) Prince Edward Island patch. Numbers of animals observed on transect are plotted at the end of each line.

TABLE 1. Stage data from 1991 aerial surveys of hooded seal pups in the southern Gulf of St. Lawrence.

Place and date	Stage				Total
	1	2	3	4	
Prince Edward Island patch					
March 15	0	68	44	14	126
17	0	34	94	38	166
19	0	36	83	71	190
Magdalen Island patch					
March 11	1	4	3	0	8
14	1	43	20	1	65
18	0	5	16	11	32
19	0	4	14	17	35

amongst the harp seal patch as early as 11 March, but surveys around the Magdalen Islands and in the southern Gulf failed to detect any other concentrations of hooded seals in the tradi-

tional whelping areas (D. E. Sergeant and W. Hoek, unpubl. data; Stenson and Wakeham 1986). The possibility of whelping elsewhere in the Gulf is unlikely, but cannot be completely discounted. Comeau (1945) found a newborn hooded seal pup near Point des Monts in the St. Lawrence River estuary in February 1898. The other most likely area for whelping to occur is in the northeastern part of the Gulf where suitable ice is sometimes found. In 1990, this area was surveyed for harp seals at which time only a single male hooded seal was observed, although pupping had already begun elsewhere. In 1991, we were informed by a helicopter pilot of a third hooded seal patch, but were unable to locate it. After our surveys had finished and the helicopter was returning to Halifax, one of us (G.B.S.) spotted a small group estimated to number <30 pups in the area that the third group had been reported.

Survey coefficient of variation declined from 28% in 1990 to less than 13% in 1991. This improvement in survey precision was achieved by increasing survey strip width and by decreasing the distance between transects. Although further reductions

in precision are desirable, it is unlikely that they can be achieved by increasing survey intensity, due to potential problems of crossing lines and double counting. In this study, we were forced to alter our protocol between the surveys of the Prince Edward Island and Magdalen Island patches in order to be able to identify harp seal beaters present within the hooded seal patch. Future studies must take this into account and be prepared to alter the survey design on short notice.

In 1990, the one observer sat in front and directed the survey. The greater visibility of the front seat combined with the difficulties in both counting and directing the survey resulted in 50% higher and much more variable counts compared with those of the rear observer. In 1991, both observers counting seals sat in the rear, and the survey was directed by a third person sitting in the front seat. As a result, less variability and smaller differences in counts were seen between observers. We recommend that a third person be used to direct surveys in future, especially in studies where the aircraft is slow moving and transect lines are closely spaced, conditions under which the potential for crossing lines is high.

We estimated that 71–93% of the pups were present on the ice during our surveys. This proportion on the ice is higher than the 44% estimated by Bowen et al. (1987b) for hooded seals at the Front and likely reflects real differences in survey conditions between the two studies. In the Gulf, hooded seals are observed on much larger pans of ice and the pupping platform appears to be much more stable than that observed at the Front (G. Stenson, pers. obs.). In this study, we assumed that no pups were in the water at the time of the survey. Fixing this parameter improved survey precision, but may have introduced some error into the estimate if this assumption was incorrect.

An estimated minimum pup production of 2006 pups is much greater than the 500–800 reported by earlier studies (D. E. Sergeant and W. Hoek, unpubl. data; Stenson and Wakeham 1986). Although the herd has probably increased since hunting ended in 1972 (Sergeant 1976), much of the difference between the two estimates reflects the fact that our study is the first quantitative survey of Gulf hooded seals.

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Functional Response and Capture Timing in an Individual-based Model: Predation by Northern Squawfish (*Ptychocheilus oregonensis*) on Juvenile Salmonids in the Columbia River

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Petersen, J. H., and D. L. DeAngelis. 1992. Functional response and capture timing in an individual-based model: predation by northern squawfish (*Ptychocheilus oregonensis*) on juvenile salmonids in the Columbia River. *Can. J. Fish. Aquat. Sci.* 49: 2551–2565.

The behavior of individual northern squawfish (*Ptychocheilus oregonensis*) preying on juvenile salmonids was modeled to address questions about capture rate and the timing of prey captures (random versus contagious). Prey density, predator weight, prey weight, temperature, and diel feeding pattern were first incorporated into predation equations analogous to Holling Type 2 and Type 3 functional response models. Type 2 and Type 3 equations fit field data from the Columbia River equally well, and both models predicted predation rates on five of seven independent dates. Selecting a functional response type may be complicated by variable predation rates, analytical methods, and assumptions of the model equations. Using the Type 2 functional response, random versus contagious timing of prey capture was tested using two related models. In the simpler model, salmon captures were assumed to be controlled by a Poisson renewal process; in the second model, several salmon captures were assumed to occur during brief "feeding bouts", modeled with a compound Poisson process. Salmon captures by individual northern squawfish were clustered through time, rather than random, based on comparison of model simulations and field data. The contagious-feeding result suggests that salmonids may be encountered as patches or schools in the river.

Nous avons préparé des modèles de comportements s'appliquant à des sauvagesses du nord (*Ptychocheilus oregonensis*) relativement à la consommation de salmonidés juvéniles, en vue d'étudier certains aspects concernant le taux de capture et le moment de la capture de ces proies (aléatoire ou « contagieux »). La densité et le poids des proies, le poids du prédateur, la température ainsi que le comportement trophique nyctéméral ont d'abord été intégrés dans des formules de prédation analogues aux modèles de réaction fonctionnelle de type 2 et 3 de Holling. Les équations de type 2 et 3 correspondent aussi bien l'une que l'autre aux données de terrain relatives au fleuve Columbia, et les deux modèles ont fourni des prévisions sur le taux de prédation pour cinq de sept dates indépendantes. La sélection d'un type de réaction fonctionnelle peut être compliquée par la variabilité des taux de prédation et la diversité des méthodes analytiques et des hypothèses concernant les équations de modèles. On s'est servi de la réaction fonctionnelle de type 2 pour étudier le rapport entre le moment choisi de façon aléatoire et le moment choisi par contagion pour la capture de proies, en se servant de deux modèles similaires. Dans le modèle plus simple, on présume que les captures de saumon sont tributaires d'un processus de renouvellement qui suit la loi de Poisson; dans le second modèle, on part de l'hypothèse selon laquelle plusieurs captures de saumon se feraient au cours de brèves « poussées trophiques », suivant un processus complexe conforme à la loi de Poisson. D'après la comparaison entre les modèles simulés et les données de terrain, les captures de saumon par chaque sauvagesse du nord étaient concentrées dans le temps plutôt qu'aléatoires. Les conclusions obtenues relativement à un modèle « contagieux », d'alimentation laissent supposer que les salmonidés peuvent être rencontrés en groupes ou par bancs dans la rivière.

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The decline of salmon stocks along the west coast of North America has stimulated the development of several mathematical and computer models aimed at understanding and managing these important fish (e.g. Peterman 1982; Monitoring and Evaluation Group 1989; Beamesderfer et al. 1990; Hsieh et al. 1991; Lee 1991). Models have been developed for salmon populations on many different spatial and temporal scales. Models of whole salmon life cycles require simulations over many years and hundreds to thousands of kilometres. The aggregative nature (multiple cohorts or age-classes) and the large temporal-spatial scale of most of these modeling

approaches preclude detailed treatment of biological interactions — competition, parasitism, and predation — that may limit fish growth or determine the survival of individual salmon.

Because of their effect on individuals, processes that occur on short time scales (seconds–minutes) and on limited spatial scales (metres) can be crucial to large-scale population dynamics, and models appropriate to these small scales are required for understanding these processes. Predator–prey interactions, for example, are usually brief encounters between individuals, although the whole sequence of predator search, prey capture, and prey handling may take several hours. Fine-scale models

can also take into account variability that may exist among individual fish (e.g. size, experience, innate behavior), which aggregative models cannot. Also, some models that describe population-level responses are dependent on individual-level trophic interactions (Hassell and May 1974; Murdoch and Oaten 1975; Green 1990).

The goal of this study was to develop a model that describes the feeding behavior of individual northern squawfish (*Ptychocheilus oregonensis*) as they prey on juvenile salmonids in the lower Columbia River. Northern squawfish have been shown to consume large numbers of migrating salmon and steelhead (*Oncorhynchus* spp.) in John Day Reservoir on the Columbia River (Rieman et al. 1991); northern squawfish have also been identified as major predators in other areas (Foerster and Ricker 1941; Jeppson and Platts 1959; Thompson and Tufts 1967; Brown and Moyle 1981). An individual-based model of the feeding behavior of northern squawfish could be used to evaluate questions about the influence of biological and physical variables and the timing (random versus nonrandom) of prey captures. The model of northern squawfish predation was developed at two levels to address these different questions: (1) daily feeding rate and (2) the specific timing of individual prey captures.

General predation models often examine the effect of prey density on the feeding rate of predators, frequently expressed as a daily predation rate. Perhaps the most prevalent approach used to model predation at this scale has been to make some assumptions about the daily allocation of time by the predator, the attack rate, and prey handling times, leading to the familiar "disc equation" for the functional response of predators to changing prey density (e.g. Holling 1959; Hassell 1978). When this approach is used, the rate of change in predation to increasing prey density has been categorized into three functional response *Types*. Specifying a functional response Type for a given predator has often been a fundamental part of predator-prey studies.

Two functional response types commonly described for vertebrates are the Type 2 (decelerating rise to an upper feeding rate asymptote) and the Type 3 (sigmoid rise to the asymptote) (Fig. 1a). Distinction of a Type 2 from a Type 3 functional response for a particular predator may be especially important in biological control programs (Murdoch and Oaten 1975; Hassell 1978) but may also be critical when considering predation mortality of juvenile salmon (Peterman and Gatto 1978; Jones 1986; Fresh and Schroder 1987; Vigg 1988). For northern squawfish, Type 2 and Type 3 functional response models were derived and compared. Besides prey density, we also incorporated the effects of predator size, prey size, temperature, and diel feeding pattern into the models.

On a shorter time scale (minutes–hours), the actual timing of salmonid captures by individual northern squawfish was modeled. Prey of many predator species occur in patches or schools, and studies on optimal foraging are often concerned with the way predators exploit these patches. Predators that search for patchily distributed prey may have behavioral strategies and energy budgets that differ greatly from predators that capture prey randomly through time. Schooling behavior may also reduce the chance that individual prey are successfully captured by a predator, through various mechanisms reviewed by Pitcher (1986). The timing of prey captures, from direct observation or back-calculated from stomach content data, has been used to test hypotheses about patch residence time, hunger thresholds, and contagious feeding events (DeAngelis et al. 1984; Marschall et al. 1989).

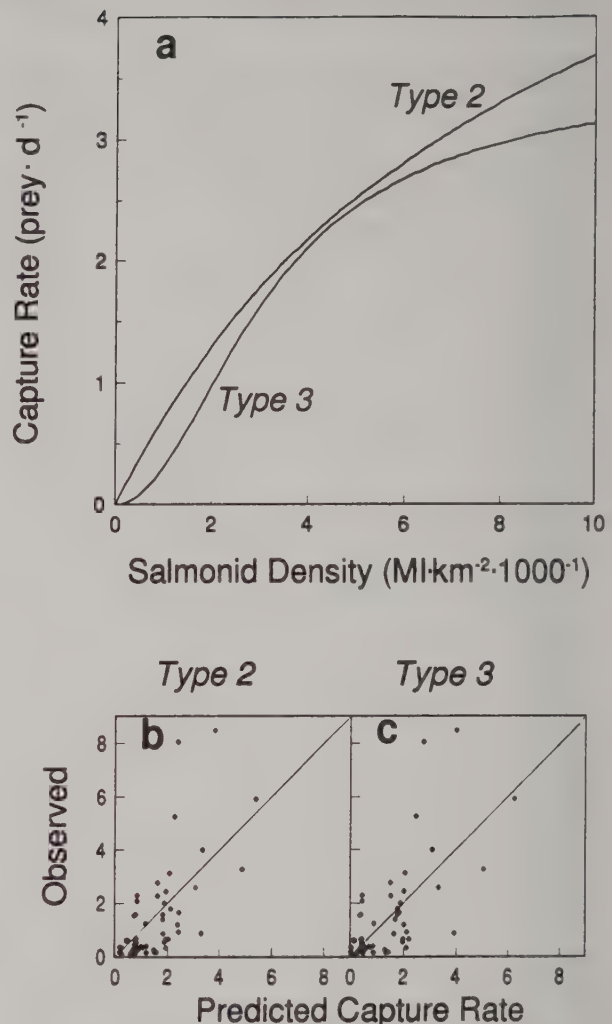


FIG. 1. Fitted functional response models for northern squawfish collected in the McNary Dam BRZ. (a) Predicted functional responses, over a typical prey density range, of a 1000-g northern squawfish eating 15-g salmon at 20°C. (b and c) Predicted and observed capture rates for the two models.

We developed and compared capture timing models for random prey captures and feeding during specific bouts (contagious feeding). Capture timing models were integrated with the functional response models in simulation programs. Simulation results were compared for effectiveness at describing observations of gut contents, and conclusions were drawn regarding the nature of northern squawfish feeding on juvenile salmonids.

Model Development

A model formulation capable of making testable predictions was required to explore the predation questions. The first part of the model development was the representation of the average daily feeding rate in terms of predator and prey characteristics. The second part was the modeling of the diurnal pattern of feeding by northern squawfish. The third part was the modeling of individual feeding events and the pattern of capture sequences through time for northern squawfish. These elements were

incorporated into simulation models and output was compared with field data.

The models developed were principally applicable to the 1-km² area immediately downriver from McNary Dam on the Columbia River, referred to as the boat restricted zone (BRZ). Model specificity for the BRZ was based on several considerations: (1) data were available from a 3-yr study (1984–86) in the BRZ for estimating parameters (Poe et al. 1991; Vigg et al. 1991), (2) prey density could be approximated in the BRZ based on dam-passage information (see Methods and Data below), (3) juvenile salmonids suffer high mortality in the McNary Dam BRZ (Rieman et al. 1991) and in other dam tailraces (Petersen et al. 1991), and (4) the diet of northern squawfish in the BRZ was dominated by juvenile salmonids (78–99%; Petersen et al. 1990, 1991; Poe et al. 1991), so diet choice could be ignored.

Mean Expected Daily Feeding Rate

The mean expected number of juvenile salmonids captured for a particular day j , which we call C_j (prey per predator per day), is a complex function of juvenile salmonid density, average weight of salmonid prey, northern squawfish weight, temperature, prey condition, turbidity, and other factors. Much of this complexity arises because daily expected feeding must include the effect of the time it takes for predators to handle the prey. We will derive two functional forms for the feeding rate that can be related to empirical data.

Consider some period within a day when fish are foraging. The foraging period can be assumed to consist of time spent searching (or waiting) for prey, t_s , and time spent handling prey, t_h :

$$(1) \quad t_s + t_h = 1.$$

The handling time includes both the time spent in capturing prey, which is relatively short and will be ignored, and the time during which the fish is relatively satiated and unlikely to be searching for prey. While a predator is relatively satiated, much of its time and energy would be spent on digestion. The time needed for digestion of an individual prey, defined here as t_d , depends on the predator weight w (grams), the prey weight S (grams), and the temperature T (degrees Celsius) since digestion rate depends on temperature (Fänge and Grove 1979). We assumed that prey handling time was related linearly to prey weight and inversely to predator weight and temperature; thus, $t_d = \alpha S/(wT)$ where α is a proportionality constant with units of day-degrees.

Beyer et al. (1988) concluded that 90% evacuation time for northern squawfish in the laboratory was exponentially related to prey weight, temperature, and predator size. The form of the evacuation equation chosen by Beyer et al. (1988, their equation 3) was similar to our formula for t_d , except that they included exponent parameters for S , w , and T . However, a linear model ($R^2 = 78\%$) explained nearly as much of the variability in evacuation as the exponential model ($R^2 = 90\%$; Beyer et al. 1988). We modeled digestion time with the linear model, rather than an exponential model, because of the relatively small improvement shown by the exponential equation in the Beyer et al. (1988) experiments, the simplicity of the linear model, and evidence from other fishes that large-sized meals are often evacuated at a linear rate (Jobling 1986).

The number of prey individuals captured during the search-ing period within a day is

$$(2) \quad C_j = \beta N_j t_s$$

where β , with units per day, is a constant that depends on the reactive distance and capture efficiency of the predator with respect to the prey, and N_j (prey per day) is a measure of the prey density migrating past the predator on a given day j .

The total time spent handling prey during the period is then

$$(3) \quad t_h = C_j t_d = [\beta N_j \alpha S/(wT_j)] t_s$$

where T_j is temperature on day j . Substituting equation 3 into equation 1, we can solve for t_s to obtain

$$(4) \quad t_s = 1/[1 + \beta N_j \alpha S/(wT_j)]$$

so that, from equation 2, the expected number of captures per day is

$$(5) \quad C_j = \beta N_j / [1 + \beta N_j \alpha S/(wT_j)].$$

Equation 5 is analogous to a Holling Type 2 functional response (Holling 1965).

Feeding rate predicted by equation 5 assumed that the rate of attack and the rate of successful capture, combined as β , were constant and did not vary with prey density, predator size, or other variables. Constant attack rates and capture success may not be a valid assumption, particularly when predators are capable of learning or “switching” between different prey types (Murdoch 1973; Abrams 1990). If the density of salmonids increases, northern squawfish may increase their rate of attack on this prey type; thus β would then be some increasing function of prey density. Variable attack rate (β') can be related to prey density N_j as

$$(6) \quad \beta' = \delta N_j$$

where δ is a fit parameter. Substituting a variable attack rate (β') for constant attack rate (β) into equation 5 produces a model that is analogous to a Type 3 functional response:

$$(7) \quad C_j = \beta' N_j / [1 + \beta' N_j \alpha S/(wT_j)].$$

Type 2 and Type 3 functional responses of northern squawfish were compared by fitting equations 5 and 7 to the same field data.

Diurnal Feeding Pattern

The capture rate, C_j , of a predator often varies diurnally (Vigg et al. 1991), and we want to include this variation in the model. This can be accomplished by adjusting the rate of prey capture within a day to reflect the diurnal variations in predator feeding activity.

Consider the day to be divided into n equal time intervals of m hours each; $t_0 \leq t \leq t_1$, $t_1 \leq t \leq t_2$, ..., $t_{n-1} \leq t \leq t_n$, where $t_n - t_0 = 1$ d. We now take into consideration the fact that the predator will be more active in feeding during certain periods within the day than it will be during others. To account for the temporal pattern in feeding that will take place within a day, define F_i ($F_i < 1$) to be the ratio of feeding activity that takes place on the interval $t_i < t < t_{i+1}$, to the daily expected feeding activity, with $\sum_{i=1}^n F_i = 1$. Feeding activity F_i may be driven

by an endogenous circadian rhythm (Thorpe 1978), or it may be more directly controlled by short-term changes in variables such as prey density or light levels (Helfman 1986).

Then, C_j times F_i is the mean expected number of prey captured for any time interval i on day j . We define

$$(8) \quad \lambda_{ji} = C_j F_i$$

where λ_{ij} is the mean expected rate of feeding per time period (m). One-hour timesteps (m) were used in simulations of northern squawfish feeding, so λ_{ij} had units prey per hour.

Timing of Prey Captures: Predation as a Poisson Renewal Process

The variable λ_{ij} is the mean number of prey captured by an individual predator during period i of a day. We want to resolve predation to the level of single capture events by individual predators. As a first approximation, assume that prey are captured at intervals described by a Poisson distribution. If the mean rate of captures is λ_{ij} , then the probability $P(t)$ of a capture occurring within some time interval $t_{i-1} < t < t_i$, measured from some initial time t_0 within this interval, is

$$(9) \quad P(t) = 1 - \exp[-\lambda_{ij}(t-t_0)] \quad t_{i-1} \leq t_0 < t < t_i.$$

Similarly, over longer time intervals within a day, this probability continues to increase as follows:

$$(10) \quad P(t) = 1 - \exp[-\lambda_{ij}(t_i-t_0) - \lambda_{i+1,j}(t-t_i)] \quad t_{i-1} < t_0 < t_i < t < t_{i+1}.$$

This scheme can be extended over multiple days, with λ_{ij} changing from day j to day $j+1$, depending on changes in prey density, temperature, and prey size. According to this model, which we call Model I, as soon as a capture occurs the predator begins to wait for another prey. This is called a Poisson renewal process and has been used to describe the feeding pattern of adult largemouth bass (*Micropterus salmoides*) preying on fish (DeAngelis et al. 1984).

Timing of Prey Captures: Predation as a Compound Poisson Process

A second model was derived to test hypotheses of contagious feeding by northern squawfish. Laboratory observations (pers. obs.) and preliminary tests of Model I suggested that northern squawfish may be capturing several juvenile salmonids during distinct feeding bouts, rather than randomly through time. If feeding occurs in bouts, prey captures would be close in time and may be modeled as a relatively fast process, compared with the slower process of interbout timing. Contagious feeding, called Model II, was modeled as a compound probability distribution (e.g. Pielou 1977) where the mean rate of feeding bouts per day (E) and the number of prey fish captured during a feeding bout (B) were both assumed to be Poisson processes. The rate of feeding bouts were modeled as a Poisson renewal process (equation 10) where $P_E(t)$ was the probability of a feeding bout occurring at time t . As described above for $P(t)$, $P_E(t)$ varied within a day according to the diel feeding activity and among days according to the prey density, temperature, and mean prey size. Models I and II were related in that both used a Poisson renewal process for capture or event generation.

During a northern squawfish feeding period, we assumed that the probability of encountering n salmon ($1 \leq n < \infty$) could be described by a Poisson process and that the number of salmon captured during a feeding bout would be proportional to the number encountered. A great many salmon might be encountered during a feeding period whereas the number of prey actually captured would be limited by predator size and physiology, and prey size. Assuming a constant proportion of captures per encounter, we modeled the probability of salmonid capture, rather than the probability of salmonid encounter. Therefore, once a feeding bout began, the probability of capturing k smolts was

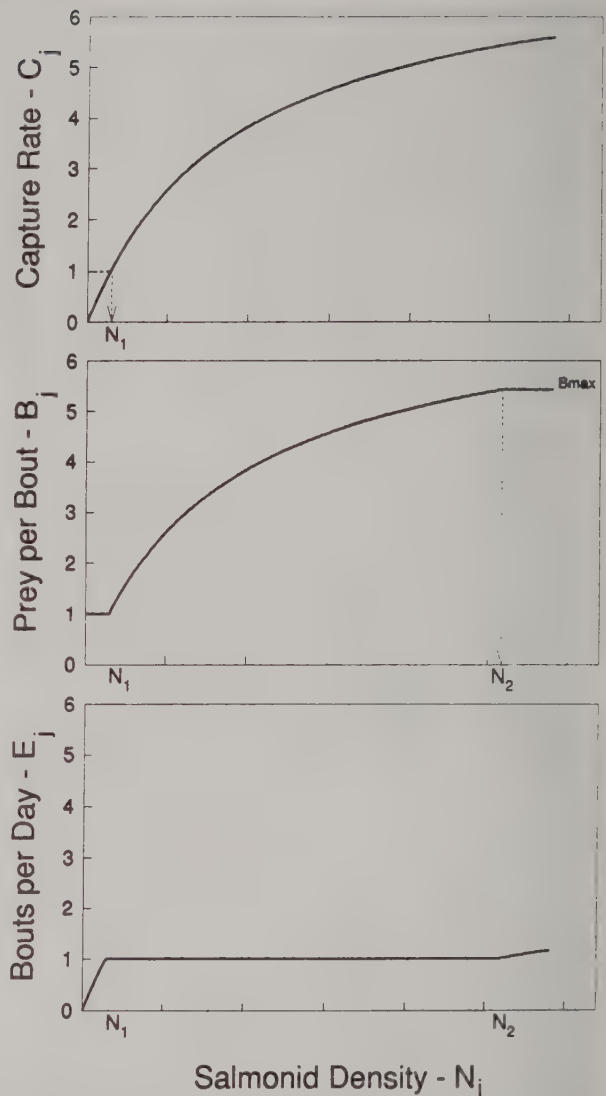


FIG. 2. Relationships in the contagious feeding model (Model II) between predicted number of salmon captures per day (C_j), prey captured per feeding bout (B_j), and feeding bouts per day (E_j). Curves are for a hypothetical individual northern squawfish. N_1 is the salmonid density where $C_j = 1$, and N_2 is the salmonid density where $B_j = B_{\max}$. See text for further explanation.

$$(11) \quad P_k(k, B) = B^k e^{-B} / k! \quad (k = 1, 2, 3, \dots, B_{\max})$$

where B is the mean number of prey captured during a bout and $B_{\max}$ is the maximum number of prey that a predator can capture per bout.

In Model II, E and B are Poisson rate parameters and had to be estimated. If feeding occurs in bouts, the mean number of captures on day j (C_j) can be described as

$$(12) \quad C_j = E_j B_j \quad (1 \geq B_j \leq B_{\max})$$

where E_j is the mean daily rate of feeding bouts and B_j is the mean number of prey captured during a feeding bout. B_j must be greater than or equal to 1 by definition, since at least one prey must be captured within a feeding bout.

We made some assumptions about how the rate of bouts and the rate of captures per bout of a general predator vary with

prey density to produce a predicted daily capture rate (equation 12; see Fig. 2). As the local density of prey increases slightly from zero, a predator would occasionally capture a prey, but since prey density is low, feeding events would not occur every day ($E_j < 1$). As prey density continues to increase, the rate of feeding events also increases until the predator is capturing at least one prey during one feeding event per day ($C_j = 1$, $E_j = 1$, $B_j = 1$; Fig. 2).

Once prey density is above some minimum (N_1 ; Fig. 2) and predators have one feeding event per day, however, we assumed that a predator's primary response to further increases in prey density would be to increase the number of captures within a bout (B_j) by taking additional prey in a meal, rather than increasing the number of feeding bouts per day. Some fishes compensate for reduced feeding frequency by requiring a larger ration for satiation or by becoming hyperphagic when food becomes available (Tyler and Dunn 1976; Grove et al. 1978; Jobling 1982; Miglav and Jobling 1989). Dill (1983) described how hunger and the rate of change in hunger might be used by fish to assess the local availability of food. A hungry fish that encounters a profitable patch of food would likely respond by feeding rapidly and taking a large meal. The alternative — more feeding bouts but a smaller-than-maximum meal — seems less likely in a variable environment. When the captures per bout have reached a maximum but prey are still available, additional feeding bouts might be added within a day until the fish is satiated. As the frequency of feeding bouts increases and the maximum number of prey are captured per bout, the capture rate for this predator would approach its upper limit (Fig. 2).

The following equations were used to describe the relationships shown in Fig. 2:

$$(13a) \quad B_j = 1 \quad C_j \leq 1$$

$$(13b) \quad B_j = C_j \quad 1 < C_j \leq B_{\max}$$

$$(13c) \quad B_j = B_{\max} \quad C_j > B_{\max}$$

$$(14) \quad E_j = C_j/B_j \quad (\text{from equation 12}).$$

Equation 13, especially 13b, assures that the number of prey captured per feeding bout increases in the same manner as the predicted daily capture rate, except at very low and high prey densities. B_j also varies with changes in predator weight, prey weight, and temperature, since these variables were used to estimate C_j . Defining B_j and E_j in this manner assured that the predicted daily rate of prey captured, C_j , was realized in the simulations through a bout feeding paradigm.

Methods and Data

Northern squawfish were collected in John Day Reservoir, on the lower Columbia River, during spring and summer of 1984–86 and July 1988. Samples were collected throughout the reservoir during 1984–86 (Poe et al. 1991; Vigg et al. 1991), while July 1988 samples were only from the McNary Dam BRZ (Petersen et al. 1990). Digestive tracts of all northern squawfish were returned to the laboratory, contents were sorted, and prey items were identified and weighed. Diagnostic bones were used to identify partially digested prey fish (Hansel et al. 1988).

The number of salmon prey captured by northern squawfish per day ("capture rate") was estimated with a technique that reconstructed the diel feeding pattern from pooled gut samples of predators (Swenson and Smith 1973; Vigg et al. 1991). Variability of the daily capture rate was estimated by bootstrap resampling of data sets (Boisclair and Leggett 1988; Petersen

et al. 1990). Based on a bootstrap analysis of feeding rate variability (Petersen et al. 1990), only daily samples with 15 or more northern squawfish (≥ 250 mm fork length, approximately > 190 g) were used to estimate capture rates. Details of collection methods, laboratory procedures, and calculation of feeding rates are reported in Hansel et al. (1988), Petersen et al. (1990), Poe et al. (1991), and Vigg et al. (1991).

The time between successive capture of juvenile salmonids (intercapture time) was computed when two or more prey fish were found in the gut of a northern squawfish. An equation from Beyer et al. (1988) was rearranged to estimate 90% digestion time, DT_{90} (hours), of each salmon:

$$(15) \quad DT_{90} = 1266D^{1.08}M^{-0.46}T^{-1.60}w^{-0.27}$$

where D is grams digested, M is meal weight (grams), T is water temperature (degrees Celsius), and w is predator weight (grams). Grams digested of a prey fish was the original weight, back-calculated from regressions of weight on diagnostic bone length (Vigg 1988), minus the weight remaining in the gut sample. Meal weight was estimated by the method of Vigg et al. (1991). Intercapture times were the differences between proximate ingestion times of prey fish in an individual predator gut.

We also wanted to compare the frequency distributions of prey in predator guts between field data and simulations. The number of juvenile salmonids observable in the guts of field-sampled northern squawfish is based on the use of diagnostic bones to identify partly digested fish (Hansel et al. 1988; Vigg et al. 1991). Since indigestible material such as bones is often evacuated from fish guts at a slower rate than digestible organic matter (e.g. Molnar et al. 1967; Kolok and Rondorf 1987; dos Santos and Jobling 1991), we estimated the retention time (RT) of prey bones in simulations as

$$(16) \quad RT = DT_{90} + \phi DT_{90}$$

where DT_{90} was the 90% evacuation time of digestible organic material (equation 15) and ϕ was a constant parameter. dos Santos and Jobling (1991) found that 2-mm plastic beads had half-lives over twice as long as natural herring prey in Atlantic cod (*Gadus morhua*) stomachs, so ϕ was arbitrarily set to 0.5. For simulations, the number of prey per predator was calculated using the retention time for prey bones.

Density of juvenile salmonids in the McNary Dam BRZ was based on the migrational index and daily water flow at McNary Dam (Koski et al. 1989). The migrational index MI was the number of smolts collected at the dam, adjusted for the volume of water that was not sampled (Fish Passage Center 1987). Migrational index for day j was converted to salmonid density N_j ($\text{MI} \cdot \text{km}^{-2} \cdot 1000^{-1}$) in the BRZ using the approach of Vigg (1988):

$$(17) \quad N_j = \text{MI (BRZ depth/flow)}$$

where BRZ depth is the average depth (10 m) of the tailrace restricted zone and flow is the daily flow (cubic metres per day) through the BRZ.

Parameters for the functional response models (equations 5 and 7) were estimated from data collected during May–August 1984–86 in the McNary Dam BRZ. For each day of sampling, northern squawfish were separated into 300-g categories, and capture rate was estimated for each weight category that contained 15 or more predators. The number of salmon captured per day, C_j (prey per predator per day), was fitted to equations 5 and 7 using least-squares nonlinear regression.

The maximum number of captures per feeding bout ($B_{\max}$), as a function of predator size, was estimated from data collected

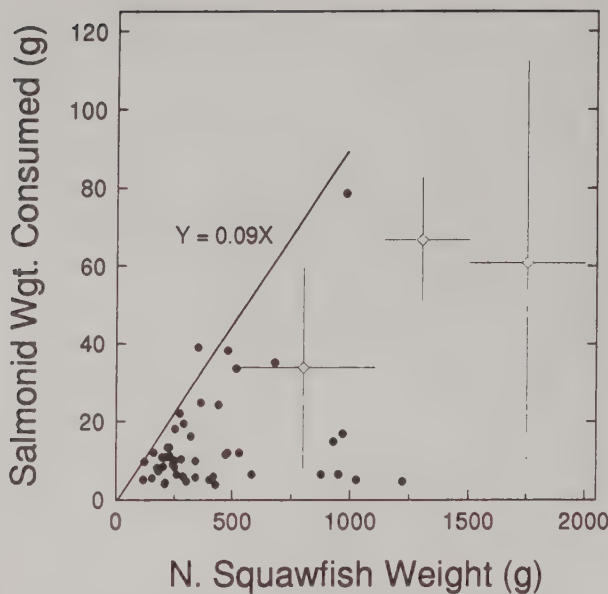


FIG. 3. Total weight of salmonids consumed by northern squawfish of various sizes. Solid circles are from tagged salmonids recovered in predator guts during a 9-h feeding experiment in the Bonneville Dam tailrace. Open diamonds were calculated from maximum rations (mean $\pm$ SD) estimated by Vigg and Burley (1991; their table 2) in laboratory experiments. The diagonal line, fit by eye to the upper edge of the Bonneville tailrace data, approximates the maximum grams that could be consumed per feeding bout by predators of different size.

within the Bonneville Dam tailrace (lower Columbia River) during July–August 1990. On two nights, batches of juvenile chinook salmon (*Oncorhynchus tshawytscha*, 6–9 g each) with coded-wire tags were released into the dam tailrace, northern squawfish fed on the tagged salmon, and the squawfish were collected within 9 h after salmon release. Northern squawfish gut contents were examined for coded-wire tags, which indicated prey capture sometime within the 9-h period. All tagged salmon found in squawfish guts were assumed to have been caught within one feeding period. Total weight consumed by an individual northern squawfish during this feeding period was estimated as the sum of back-calculated salmonid weights.

As many as 15 tagged salmonids were captured by an individual northern squawfish during the 9-h experiment near Bonneville Dam. The maximum weight of salmonids captured during this experiment was about 9% of the predator weight (Fig. 3). Maximum prey weight consumed during the Bonneville experiment was greater than the maximum prey weight calculated from the mean daily ration (grams prey per gram predator per day) for northern squawfish that was estimated by Vigg and Burley (1991) (see Fig. 3). The upper limit of the error bars for the Vigg and Burley weight groups (Fig. 3), however, suggests that individual northern squawfish in the laboratory experiments likely had rations as high as 9% of their body weight. Northern squawfish may also have gorged themselves (hyperphagy) when exposed to the high-density patches of salmonids released into the river (*sensu* Jones 1986; Miglav and Jobling 1989).

The maximum number of captures per feeding bout was defined by the upper limit of a plot of total weight consumed versus predator weight (Fig. 3; also see Knight and Margraf

TABLE 1. Parameters estimates (SE) and summary statistics of Type 2 and 3 functional response equations fitted to northern squawfish data. *Fitted parameter significantly different from zero (*t*-test, $P < 0.05$).

Parameter or statistic	Functional response form	
	Type 2 (equation 5)	Type 3 (equations 6 and 7)
α	156.7* (55.4)	309.6* (49.7)
β	0.8* (0.3)	—
δ	—	0.34* (0.1)
N	59	59
R^2 (%)	45	46
Residual average (SD)	-0.1 (1.3)	0.1 (1.3)

1982). The maximum number of captures per feeding bout, $B_{\max}$, was

$$(18) \quad B_{\max} = \tau(w/S)$$

where τ is the slope of the diagonal line in Fig. 3, w is predator weight, and S is average prey weight.

Simulations

The probability functions $P(t)$, $P_E(t)$, and $P_k(k, B)$ defined above were used in computer programs along with a pseudo-random number generator to calculate actualized sequences of predation events by individual northern squawfish. In each simulation, individual predators (≥ 190 g) were first chosen randomly from a distribution of weights representative of the McNary Dam BRZ on a particular day — the “target” day. Predation by individual northern squawfish was simulated for five consecutive days, during which temperature, prey density, and prey size changed from day to day. The fifth day of the sequence was the target day, and simulation output was accumulated for this day. A 5-d run was used to be sure initial conditions did not affect the target day output.

Simulation inputs were date-specific predator weight distribution, water temperature, mean prey weight, and juvenile salmonid density at McNary Dam. To generate model output for a target day, 25 simulations were run, with 100 randomly chosen northern squawfish simulated per run. Mean capture rates were the average of the 25 runs, while intercapture time of prey per predator distributions were based on all simulated individuals ($n = 2500$). Variation in the number of prey captured per day was generated by sampling normal distributions of the functional response parameters; nominal parameter values and SE's in Table 1 were used to describe the sampled distributions.

During each hour of the 5-d simulation period, capture rate was predicted and the occurrence of a prey capture or feeding bout was determined by comparing $P(t)$ for Model I or $P_E(t)$ for Model II with a uniform random number. In Model I, the exact time of a prey capture was a random time within the hour. For Model II, a feeding bout required determining the number of prey captured k (equation 11) and the capture times of the k prey. Times of prey captures were determined by adding each of $k - 1$ intercapture times to the time for the first capture, which was randomly selected within the hour. Each of the $k - 1$ intercapture times in a bout was a fraction of an hour, equal to $1/\text{mean number of prey captured during a bout } (B_j)$ for this day. On a given day, for example, a predator having a mean capture rate of two salmon per day and one feeding bout per day had intercapture times of 0.5 h ($1/2$) within a feeding bout.

Simulations were run for seven target days during July 1988, when field data had been collected from the McNary Dam BRZ

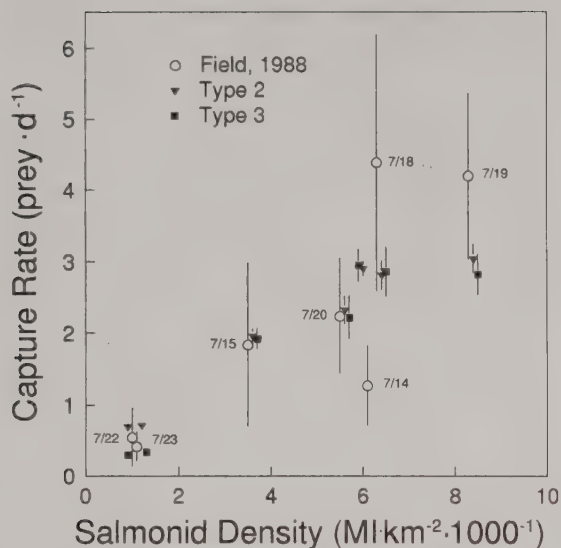


FIG. 4. Observed and predicted number of juvenile salmonids captured by northern squawfish ($\pm$ 95% confidence intervals) for seven days in July 1988. Predicted capture rates (offset slightly for clarity) were estimated using fitted Type 2 and 3 functional response equations. Capture rates were plotted as a function of juvenile salmonid density; numbers beside symbols are dates.

(Petersen et al. 1990). Field collection and laboratory methods were the same for all samples (1984–88) to ensure comparability (see Methods and Data above). Data from the July 1988 target days had not been used during the parameter estimation process.

Model Evaluation and Selection

Two functional response models (Types 2 and 3) and two capture timing models (Models I and II) had to be evaluated. The functional response model was selected first, followed by evaluation of the capture timing models.

The regression statistics were similar for Type 2 and 3 functional response equations fitted to field data. The fit parameters of both equations were significantly different from zero, explained variation was equal, and mean residuals were only different by sign (Table 1). The shapes of the fitted functional response curves for an average-sized predator (1000 g) were very similar (Fig. 1a). At very low capture rates, the Type 3 functional response may have fit the data better than the Type 2 equation, but the difference was slight (Fig. 1b and 1c). Neither model accurately predicted the two highest observed capture rates. Several Type 3 functional response equations with more complex attack functions (β' , equation 6) were examined (results not shown), with little improvement in regression fits over the simpler equations.

We also tested the ability of Type 2 and 3 functional response equations to predict the mean capture rates for July 1988 days not included in the data set used for parameter estimation. For each test day, the mean number of prey captured per day from the Type 2 and 3 equations was compared with the measured capture rates for an "average" predator (Fig. 4). Northern squawfish captured juvenile salmonids during July 1988 at rates of 0.4–4.4 prey·predator⁻¹·d⁻¹ (Fig. 4). The predicted capture rates were within the 95% confidence interval of field estimates on five of seven days for both Type 2 and 3 functional responses

(Fig. 4). Performance of the two models was nearly identical except at the lowest and highest prey densities (Fig. 4).

Both models underpredicted the number of salmon captured per day by northern squawfish for 18 and 19 July 1988, when the observed prey density and predation rates were relatively high (Fig. 4). For these days, predicted daily capture rates were roughly 35% below the observed capture rates of 4.4 and 4.2 prey·predator⁻¹·d⁻¹ (Fig. 4). It may not be too surprising that high capture rates were not well predicted by the models, since the 1984–86 data used to estimate model parameters had only one day (14 July 1986) with a high capture rate and few days with intermediate predation rates (Vigg 1988). The regression fits to equations 5 and 7 were therefore weighted toward the low capture rates, which were more accurately predicted. Also, field estimates of consumption rates from other fish species are known to be widely variable (Smagula and Adelman 1982; Rice and Cochran 1984; Amundsen and Klemetsen 1986; Boisclair and Leggett 1988; Hodgson et al. 1989), making precise model predictions difficult.

The number of salmon captured daily by northern squawfish was poorly predicted for 14 July 1988; simulated capture rate was about 2.9 prey·predator⁻¹·d⁻¹ for both models whereas the field value was 1.3 prey·predator⁻¹·d⁻¹ (Fig. 4). The observed predation rate on 14 July 1988 was atypical with respect to the prey density on that date. Juvenile salmonid density on 14 July 1988 was 6.1 MI·km⁻²·1000⁻¹, a moderately high prey density that caused the high predicted capture rates in simulations for this date. Prey density on 18 July 1988, by comparison, was only slightly higher than on 14 July (6.3 MI·km⁻²·1000⁻¹), other conditions were similar on both days, but the observed capture rate was almost four times as high on 18 July compared with 14 July (Fig. 4).

Based on the fit characteristics and independent predictions of the number of salmon captured per day, either functional response equation could be used in the further simulation studies. Differences in predicted capture rates between the two functional responses were minor. Because of its somewhat simpler form, the Type 2 functional response (equation 5) was used in the simulations concerning capture timing. The difficulty of selecting between disparate functional responses is considered in the Discussion.

Capture timing models (I and II) were evaluated with two types of simulation output: (1) frequency distributions of intercapture times and (2) frequency distributions of the number of salmon per squawfish gut. The time intervals between captures — the intercapture times — can be used to test specific hypotheses about feeding behavior (DeAngelis et al. 1984; Marschall et al. 1989). Using an individual-based modeling approach with largemouth bass, DeAngelis et al. (1984) described how an observed distribution of intercapture times would differ from a theoretical, Poisson-generated distribution. Specific deviations from the theoretical distribution (e.g. skewness, bimodality) could indicate contagious feeding, hunger thresholds, or diurnal feeding. We also used intercapture time distributions to compare model predictions with field data. Predator feeding behavior may influence both the observed time sequence of capture (intercapture times) and the number of prey observable per predator, making it necessary to examine both types of distributions (DeAngelis et al. 1984).

Intercapture time distributions and prey per predator distributions are presented for simulations of three days (15, 19, and 23 July 1988) that had large sample sizes and that spanned the range of observed capture rates (Fig. 5 and 6). Frequency dis-

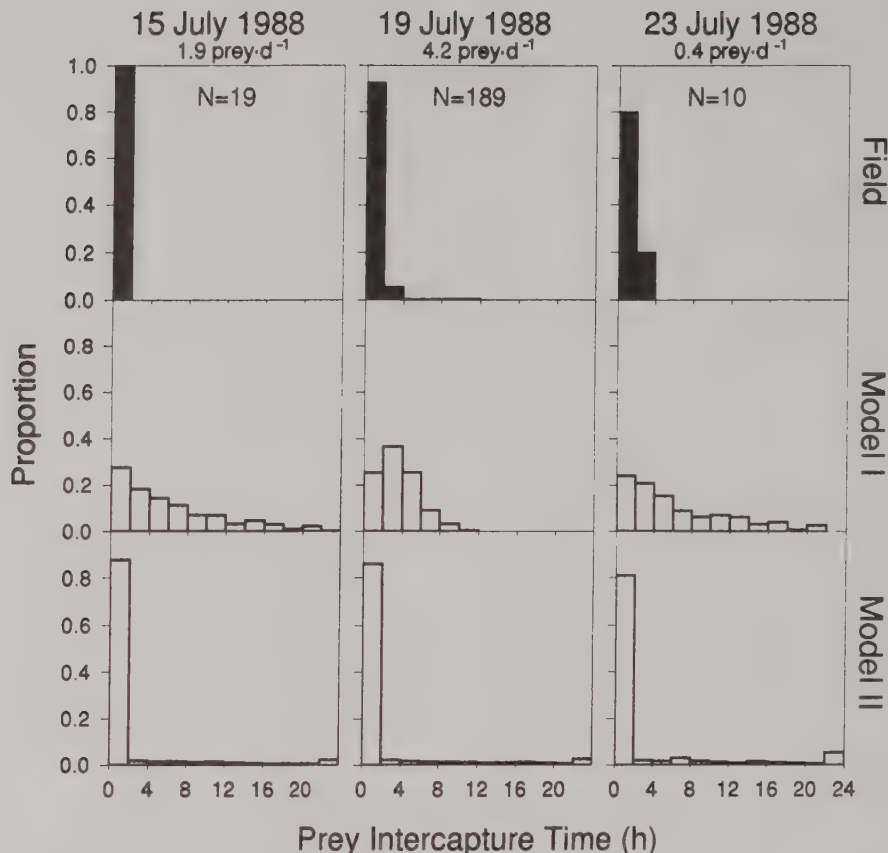


FIG. 5. Frequency distributions of intercapture times for northern squawfish feeding on salmonids on three days in July 1988. Distributions are shown for field samples, simulation of random captures (Model I), and simulation of contagious feeding (Model II). N is the total number of intercapture times on each day from the field samples.

tributions from the other four simulation dates had similar patterns to those presented.

All frequency distributions of intercapture times from northern squawfish collected in July 1988 were strongly skewed toward short time intervals. The median intercapture time was 0.2 h on 19 July, 0.4 h on 15 July, and 1.0 h on 23 July (Fig. 5).

Simulations of northern squawfish predation with Model II, which included contagious feeding, produced intercapture time distributions that were more similar to the field distributions than Model I output (Fig. 5). In the 23 July simulation, the proportion of intercapture times decreased smoothly from short to long times using Model I, while Model II produced a frequency of short intercapture times. The Model II frequency distribution was similar to the left-skewed distribution from the field data, although only 10 intercapture times could be measured from gut samples.

For the 19 July 1988 simulations, when the number of salmon captured per day was high, the intercapture time distribution from Model II and the field were nearly identical (Fig. 5). The strongly skewed intercapture time distributions observed in the field data from 19 July (Fig. 5) would be characteristic of bout feeding, with most captures being close in time followed by relatively long periods between bouts. Intercapture time distri-

butions for 15 July were also similar between Model II output and the field.

The frequency distributions of prey per predator produced by Model II were qualitatively more similar to the field distributions than were the results from Model I (Fig. 6). For 23 July, the simulated distributions of the juvenile salmonids in northern squawfish guts were nearly identical for both models, and the match to the field data was very good (Fig. 6). None of the individual northern squawfish from the field had more than four smolts in its gut, since the capture rate was quite low for this day and prey evacuation rate would have been high. On 15 July, the prey per predator distributions were nearly identical between the field and Model II (Fig. 6). The range of prey numbers found in northern squawfish guts was much greater on 19 July (Fig. 6), when the feeding rate was much higher; as many as 12 prey were found in a northern squawfish gut collected on 19 July. The number of prey per predator gut from the Model II simulation was qualitatively more similar to the field data than the Model I output.

Model II was chosen for further study, based on the greater similarities of the field and Model II distributions of intercapture times and prey per predator. All simulations below were performed using the Type 2 functional response and the contagious feeding model, Model II.

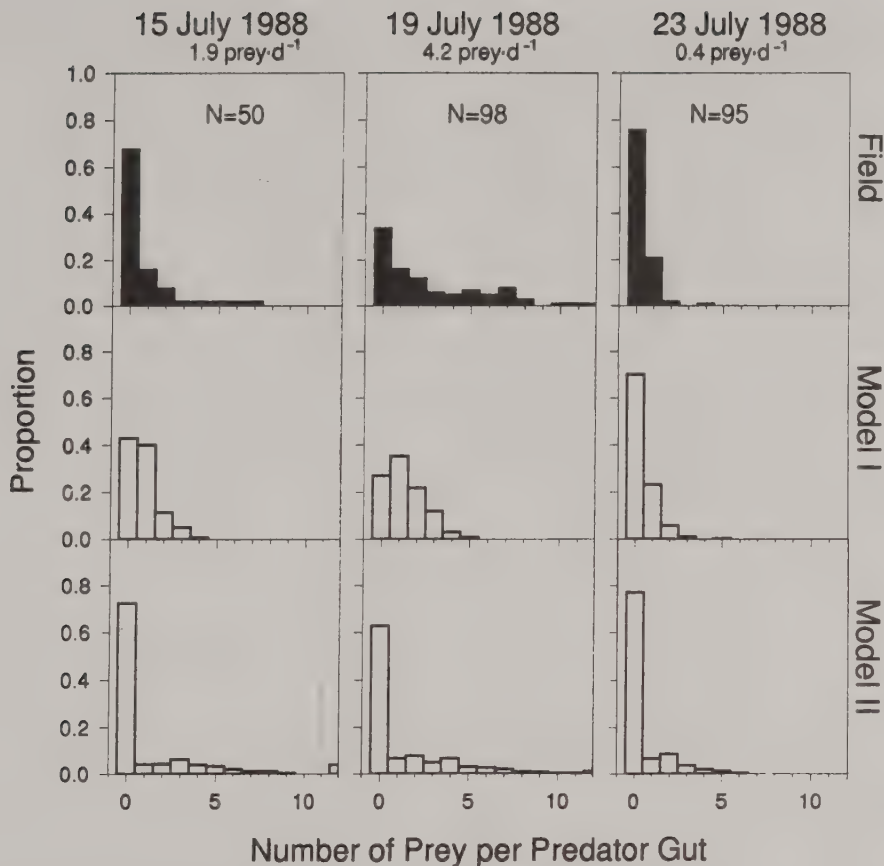


FIG. 6. Frequency distributions of the number of salmonids per northern squawfish gut on three days in July 1988. Distributions are shown for field samples, simulation of random captures (Model I), and simulation of contagious feeding (Model II). N is the total number of predators on each day from the field samples.

TABLE 2. Sensitivity of the northern squawfish simulation model to variation in Type 2 functional response parameters. Parameters were varied ± 1 and ± 2 SE/mean estimate (see Table 1). Temperature, prey density, mean prey weight, and the predator weight distribution from 19 July 1988 were used as input for these sensitivity simulations. Capture rate ($\text{prey} \cdot \text{predator}^{-1} \cdot \text{d}^{-1}$) was the tested simulation output.

Parameter		Output sensitivity	
Name	Change (%)	Capture rate (SE)	Capture rate change (%)
	Nominal	3.0 (0.4)	
α	+35	2.9 (0.4)	-3
	-35	3.5 (0.4)	17
	+70	2.5 (0.3)	-17
	-70	4.3 (0.5)	43
β	+38	3.6 (0.5)	20
	-38	2.6 (0.3)	-13
	+76	3.8 (0.4)	27
	-76	1.4 (0.2)	-53

Model Sensitivity

Internal Parameter Sensitivity

The nominal values of internal parameters α , β , ϕ , and τ

were varied to investigate the sensitivity of the model output. Because of the small number of internal parameters involved, no attempt was made to study the possible effect of parameter covariance (e.g. Bartell et al. 1986).

Parameters (α and β) in the Type 2 functional response equation were increased and decreased by one and two times their respective coefficients of variation (Table 1). Model output for these parameter changes was the number of salmonids captured per day by northern squawfish. Capture rate from the simulations was equally sensitive to changes in α and β (Table 2). In no case was the percent change in capture rate greater than the percent change of an internal parameter. Reducing the nominal value of α by 70%, for example, caused the daily capture rate to increase by only 43%, to 4.3 salmon captures per day (Table 2). These results indicate that the simulation model was fairly robust with respect to estimation of the internal parameters.

Parameters for the contagious feeding part of the model (ϕ and τ) would be expected to influence the distributions of intercapture times and the number of prey per predator gut, rather than the capture rate. Since no variability estimates were available for ϕ and τ , they were varied by arbitrary, but reasonable, amounts (Fig. 7). Only distributions of the number of prey per predator gut are shown for ϕ and τ sensitivity (Fig. 7); intercapture time distributions were somewhat less sensitive.

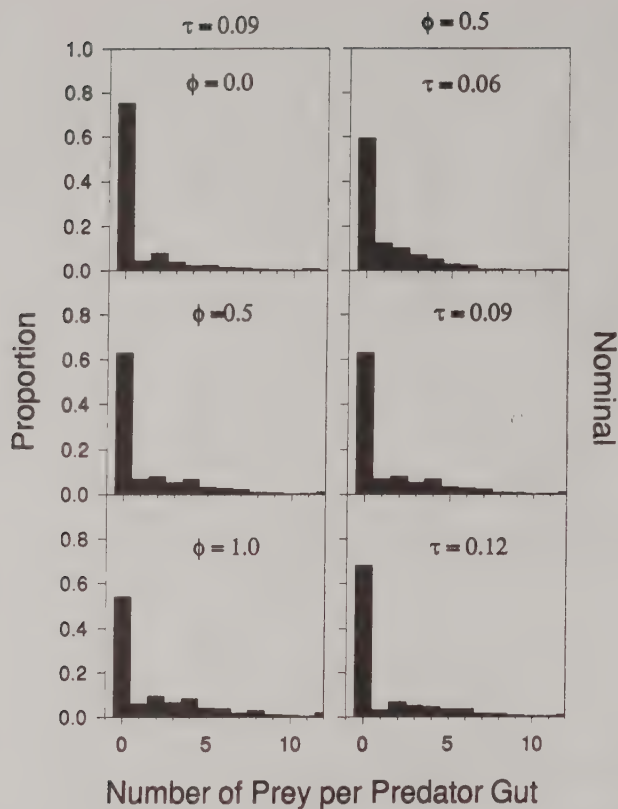


FIG. 7. Sensitivity of the number of salmonids per northern squawfish gut to variation in the bone retention time (ϕ) and the maximum captures per bout (τ) parameters. Parameters were varied independently.

Increasing ϕ , which increased the time salmonid bones were retained in a predator's gut, caused the distribution of prey per predator gut to shift gradually toward the right and fewer predators had zero prey in their guts (Fig. 7). Increasing τ , which increased the maximum number of prey a predator could capture during a feeding bout, caused a higher proportion of the predators to have zero prey in their guts and the frequencies of predators with prey became flattened and extended toward larger prey numbers (Fig. 7). Note that increased retention time of diagnostic bones or a decrease in the maximum captures per bout would produce a simulation distribution of prey per predator gut that more closely matched the observed field data for 19 July 1988 than the original simulation with Model II (Fig. 6). Refined estimates of the bone retention and maximum capture parameters might improve model performance.

Diel Feeding Pattern

We simulated five diel feeding patterns (Fig. 8) and compared capture rates and capture timing. During an earlier study (Vigg et al. 1991), the diel feeding pattern of northern squawfish differed between the McNary Dam BRZ and other areas of John Day Reservoir. In the BRZ, northern squawfish feeding rate was high from midnight to 04:00, and another feeding peak occurred from 06:00 to 10:00; feeding was lowest during midday and afternoon (Fig. 8). In the reservoir pool, feeding rate gradually increased from early morning to midday and was lowest during evening and night (18:00–04:00).

The mean number of salmon captured per day by northern squawfish did not differ between the nominal, constant, and

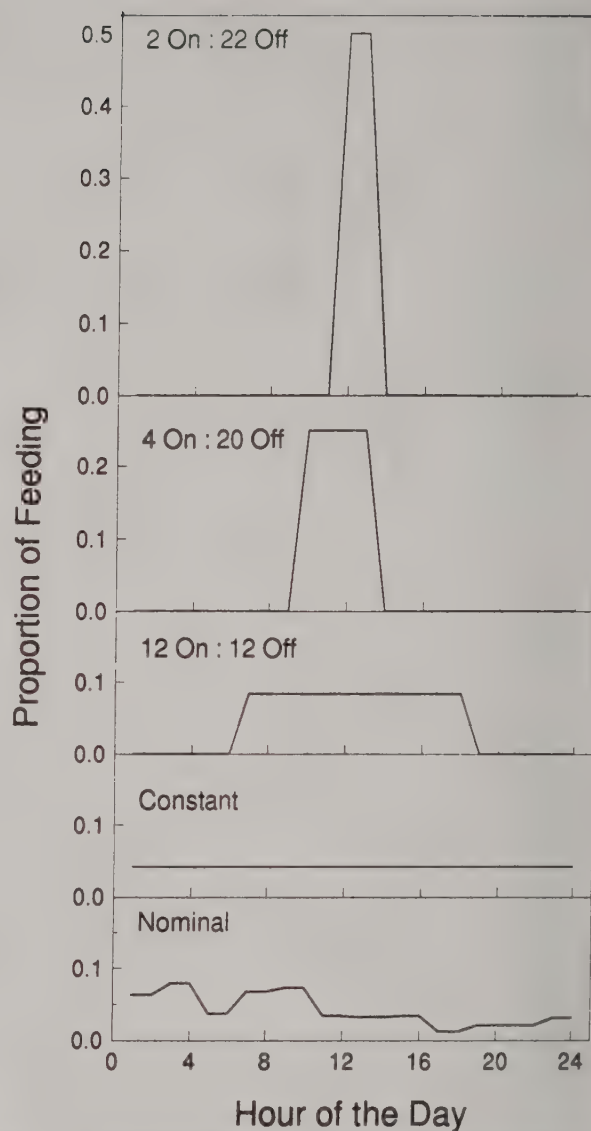


FIG. 8. Five diel feeding patterns of northern squawfish that were compared in simulations (see Table 3). The nominal feeding pattern was estimated for the McNary Dam BRZ by Vigg et al. (1991); other patterns are hypothetical.

TABLE 3. Northern squawfish capture rate (prey·predator⁻¹·d⁻¹) for simulations with different diel feeding patterns. See Fig. 8 for diel feeding patterns that were simulated. Other input: predator weight distribution from 19 July 1988, 20°C, prey weight 6 g, and prey density 6.0 MI·km⁻²·1000⁻¹. Table values are averages of 25 replicate simulations (100 individual fish in each simulation).

Diel pattern	Mean capture rate (SE)	Median intercapture time (h)
Nominal	3.0 (0.4)	0.24
Constant	3.0 (0.4)	0.23
12 on : 12 off	3.0 (0.4)	0.22
4 on : 20 off	2.8 (0.3)	0.23
2 on : 22 off	2.5 (0.3)	0.23

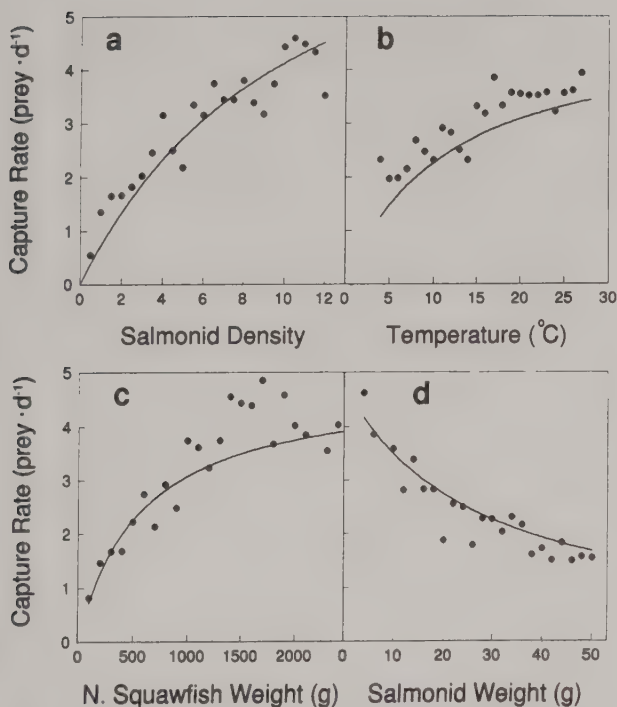


FIG. 9. Simulations of the capture rate of northern squawfish for juvenile salmonids as functions of (a) salmonid density, (b) temperature, (c) northern squawfish weight, and (d) salmonid weight. Points are means for 100 simulated predators and solid lines are the predicted capture rates from the Type 2 functional response model. Nominal conditions for simulations: 1000-g northern squawfish, 20°C, 15-g salmonid prey, and salmonid density of 6.0 $\text{MI} \cdot \text{km}^{-2} \cdot 1000^{-1}$.

12-h on : 12-h off diel feeding patterns (Table 3). Reducing the number of hours when juvenile salmonids could be captured to a 4-h period caused a 7% decline in the rate of salmon eaten, and a 2-h period caused the rate to decrease 17% (Table 3). The shorter periods of feeding opportunity reduced the number of feeding bouts that were possible for a predator during a day, therefore reducing the average capture rate per predator. The median intercapture time did not differ among the simulations, indicating that capture rate during a feeding bout was not affected by alteration of the diel feeding pattern.

Other Model Parameters

Simulations were run to demonstrate the response of northern squawfish capture rate to change in juvenile salmonid density, temperature, predator weight, and mean salmonid weight (Fig. 9). In each simulation, one variable was incremented through a representative range, while other variables were held constant.

Migrational indices of juvenile salmonids at McNary Dam usually have a spring and a summer peak (Poe et al. 1991), although salmon density also fluctuates considerably from day to day, reflecting, in part, the passage of both wild and hatchery-reared salmon. In sensitivity simulations, juvenile salmonid density was varied from 0.1 to 12.0 $\text{MI} \cdot \text{km}^{-2} \cdot 1000^{-1}$ (Fig. 9a), which reflects the usual range of prey density through spring and summer. The simulated capture rate for a 1000-g northern squawfish at 20°C increased in a Holling Type 2 functional form to about 4 $\text{prey} \cdot \text{predator}^{-1} \cdot \text{d}^{-1}$ at high juvenile salmonid density.

Water temperature in the lower Columbia River can range from about 3°C during winter to over 20°C in late summer and fall. Temperature usually remains low until June or July. The simulated number of salmon captured per day by northern squawfish increased rapidly when temperature varied between 3 and 12°C, but feeding rate increased more slowly when the temperature was above 12°C (Fig. 9b). Vigg and Burley (1991) observed a somewhat faster increase in northern squawfish maximum consumption rate as a function of temperature than was predicted by our model (Fig. 9b). For example, they measured daily rates of 0.5 $\text{prey} \cdot \text{predator}^{-1}$ at 8°C and 7.0 $\text{prey} \cdot \text{predator}^{-1}$ at 21°C, compared with our predicted 1.7 and 4.0 $\text{prey} \cdot \text{predator}^{-1}$ at similar temperatures. Part of this difference in results may have been caused by the larger mean predator size (approximately 1300 g) used by Vigg and Burley (1991) and by the fact that consumption was not significantly affected by predator size in their laboratory experiments.

The size of northern squawfish is an important consideration in feeding behavior. The weight range of northern squawfish often varies greatly depending on sample location and time of year (USFWS, unpubl. data). For example, the weight of squawfish collected within the McNary Dam tailrace during July 1988 ranged from 170 to 1682 g. The simulated number of salmon captured per day increased rapidly as squawfish size increased from 200 to about 1000 g (Fig. 9c). Mean capture rate increased more slowly for northern squawfish greater than 1000 g, compared with smaller predators (Fig. 9c).

The mean size of juvenile salmonids passing McNary Dam varies throughout the spring and summer as different species and stocks migrate downriver (Vigg 1988; Koski et al. 1989). In April, salmonids are relatively large (>40 g), as yearling chinook, coho (*O. kisutch*), and sockeye salmon (*O. nerka*) and yearling steelhead (*O. mykiss*) pass McNary Dam. Average salmonid size decreases during May and relatively small (<20 g) subyearling chinook salmon are the most common stock during June. In July and August, mean salmon size gradually increases as summer and fall chinook salmon migrate through the lower Columbia River. Most juvenile salmonids encountered by northern squawfish during a brief period (days) would be roughly the same size, so a mean salmon weight was used in the models rather than a distribution of prey sizes. The simulated number of salmon eaten by northern squawfish decreased steadily (Fig. 9d) as prey size was changed from small to large (<10–50 g). Simulation results in Fig. 9d did not reflect prey size limitations because a 1000-g predator can capture several 50-g salmon during a feeding bout (Fig. 3). Prey size thresholds were, however, included in other simulations by limiting the maximum weight of prey that could be consumed per feeding bout (B_{max} , equation 18).

Of all the input variables, the number of salmon captured per day was especially sensitive to changes in salmonid density and the size of individual northern squawfish (compare Fig. 9a–9d). Over short periods (days), prey density and, perhaps, the size distribution of northern squawfish could change drastically, causing a concurrent change in the capture rate at a particular site. Temperature and prey size show seasonal changes and thus would not cause large day-to-day changes in predation rate. The range of successive point estimates for capture rate was relatively large for all the simulations (Fig. 9), reflective of the variability of the functional response parameters (Table 1).

Discussion

Identifying the functional response of a predator to changing prey density has often been a difficult task. Comparative curve

fitting, least-squares analysis of linear models, and logit analysis have been used to distinguish functional response types, but these methods were often developed for application to laboratory predation experiments (e.g. Williams and Juliano 1985; Trexler et al. 1988). A commonly used method for identifying the functional response type in fish predation studies has been to examine the graph of consumption rate or percent prey mortality versus prey density (e.g. Ruggerone and Rogers 1984; Fresh and Schroder 1987). Highly variable predation rates measured for fish in natural habitats can make graphical distinction of functional response types difficult (Peterman and Gatto 1978; Fresh and Schroder 1987). We chose a Type 2 functional response for simulating northern squawfish feeding rate although the Type 3 equation that we tested predicted similar capture rates and had similar fit statistics.

Both Type 2 and 3 functional responses have been observed in field and laboratory studies of predaceous fish (Ware 1972; Swenson 1977; Peterman and Gatto 1978; Ruggerone and Rogers 1984; Fresh and Schroder 1987; Lyons 1987). Ruggerone and Rogers (1984) concluded that Arctic char (*Salvelinus alpinus*) had a Type 2 functional response to sockeye smolt density in the Little Togiak River, Alaska. Arctic char predation rate was best explained by a model that included migration densities of sockeye salmon, salmon weight, and char size. In three of four experiments, large rainbow trout (*O. mykiss*) and coho salmon had a Type 2 functional response when feeding on juvenile chum salmon (*O. keta*), but predation was Type 3 in the fourth experiment (Fresh and Schroder 1987).

Separating different functional response types may also depend on the degree of data aggregation used during analysis. Vigg (1988) concluded that the functional response of "average" northern squawfish was best fit by an exponential saturating curve, analogous to a Type 3 functional response. We used the same raw data as Vigg (1988) but partitioned the data into weight classes rather than combining all individuals from a given day. Stratification of predators into weight classes was designed to provide an approximate "individual" functional response. The partitioned data did not allow us to easily identify a functional response type, although the aggregate predation rates were fairly sigmoid when plotted against prey density (Vigg 1988). The aggregate functional response of a population of predators may be Type 3 whereas individuals show a "modified" Type 2 response (Carl Walters, cited in Peterman 1977 and Vigg 1988). Individual predators would have different attack thresholds of prey density; below this threshold, prey would not be attacked. Samples of predators collected at times of low prey density might include some individuals above their attack threshold and some below, but the sample predation rate would be greater than zero. The functional response of sampled predators over the range of prey densities would be a Type 3.

Besides variable data and incompletely developed methods for comparing different models, functional response analyses are often dependent on assumptions in the specific equations, such as the Type 2 equation that we used. Abrams (1982, 1990) has shown how the adaptive behavior of predators can cause the functional response to be quite variable. Abrams (1982, 1990) changed some assumptions of the disc equation and allowed parameters for the attack rate, handling time, and the proportion of time spent foraging to vary as a function of prey density, rather than remain constant. Functional response using these modified assumptions was often consistent with a Type 2 form, but the shape of the response could be significantly different from the predictions produced by the constant-parameter disc equation. Using a constant-parameter model for a preda-

tor's functional response may limit the predictive ability of the model, especially when a predator can quickly adapt its behavior or alternative prey are available (Abrams 1990). The traditional Type 1, 2, and 3 classification of functional response may be inadequate when modeling complex or multispecies systems.

It is not clear whether the functional response models that we derived would be applicable to predation in the body of Columbia River reservoirs where several alternative prey are eaten by northern squawfish (Poe et al. 1991). Our models were fit to data from the McNary Dam BRZ where northern squawfish preyed almost exclusively on salmonids (Poe et al. 1991) and, thus, a Type 2 response might be expected (Murdoch and Oaten 1975). At midreservoir locations, crayfish and sculpins were the dominant prey types of northern squawfish, with juvenile salmonids being only 8–19% of the diet by weight (Poe et al. 1991). In the midreservoir, northern squawfish may prefer to feed on resident prey types such as crayfish, whose abundance is not highly variable from day to day. Predation on migrating juvenile salmonids, whose density likely fluctuates daily or hourly at a given point in the reservoir, may be more sporadic, with predators occasionally "switching" to feed on salmon when local salmon density increases. With alternative prey available, the functional response of northern squawfish feeding on salmonids may be Type 3, since predators take longer to learn that salmon are present at low prey density than at high prey density (Murdoch and Oaten 1975). Throughout John Day Reservoir, northern squawfish that had salmonids in their guts had a higher density (CPUE) and a greater degree of aggregation than predators without salmonids (J. H. Petersen, unpubl. data), suggesting a change in foraging behavior by northern squawfish when they encountered salmon prey.

Either predator search behavior or patchiness of salmon prey could influence the rate of prey encounter and the timing of prey captures. Two models for capture timing were compared: one used a simple Poisson renewal process that assumed prey capture events were equally probable during each hour of the day, and the other assumed discrete feeding events. One of the main conclusions from comparison of the two models was that a simple Poisson renewal process was unable to accurately describe the observed numbers of prey per predator gut and the large proportion of very short intercapture times. Failure of the simpler model to match observed patterns suggested a temporal clustering of prey captures. The second model, which used a compound Poisson distribution, was able to describe this clustering. Note that our selection of a Type 2 functional response model had no significant effect on our capture timing conclusions, since both functional response models predicted similar capture rates.

Direct and indirect evidence indicates that some fishes feed in bouts. Grove et al. (1978) trained rainbow trout to activate a demand feeder that delivered a measured quantity of pelleted food. Rainbow trout feeding activity recurred at 5-h intervals during the dark when a winter photoperiod was used; fish also showed regular feeding bursts at intervals of 5–6 h in constant light conditions. Colgan (1986) stated that pumpkinseed (*Lepomis gibbosus*) feeding behavior was well described by a highly skewed gamma probability distribution, although no data were presented. Bout feeding behavior can also be inferred from data on the time required for satiation and the rate at which hunger returns (e.g. Beukema 1968; Brett 1971; Grove et al. 1978). A short satiation time combined with a comparatively long time for appetite recovery (usually associated with gastric emptying rate) suggests that, given available prey, fish feed

rapidly to satiation but the motivation to feed again may be slow to return.

Temporally clustered prey captures by northern squawfish probably mean that juvenile salmonids were encountered as "patches" or schools. Other studies have shown that predators are able to quickly assess changing density of prey patches and rapidly adjust their feeding rates (e.g. Krebs and Kacelnik 1984; Lucas 1990). Behavior of either prey or predators could cause northern squawfish to encounter salmon as patches. The movements of individual northern squawfish certainly expose them to different prey densities, water velocities, and other conditions that affect prey encounter rate and perhaps capture success. Faler et al. (1988) radiotagged 23 northern squawfish in the McNary Dam tailrace during 1984–85 and monitored movements during spring and summer. Sixteen tagged fish traveled over 2.5 km away from the dam and left the tailrace; 12 of these fish subsequently returned to the tailrace. Faler et al. (1988) attributed these movement patterns to water discharge rate and velocity — fish moved out of the tailrace during times of high water discharge and returned when discharge decreased. Juvenile salmonid behavior, such as schooling, nearshore migration, or aggregation in slackwater areas, would also cause patchiness and, perhaps, discrete feeding bouts by northern squawfish.

The apparent patchiness of prey raises some questions concerning the accuracy of the modeling of capture rate, which was based on a daily migrational index N_j . At any given time the actual concentration of prey near a northern squawfish may deviate substantially from the average daily density. For example, most juvenile salmonids migrate past McNary Dam during the night (Brege et al. 1988). Depending on the ability of individual squawfish to feed efficiently in high or low prey concentrations, the resultant feeding could differ from the model predictions since the parameter values of the functional response equations were derived from average daily migrational indices, which may not reflect the densities of prey on a shorter time scale. Hence, the discrepancies between data and model predictions for some days may be due to the way in which the salmonid densities varied within those days.

Another model assumption was that squawfish do not interact with each other in any way that would affect their capture success, either by territoriality that would lead to some fish experiencing higher prey densities than others or by interference competition that would lower the feeding effectiveness of some predators. Appropriate data were lacking for inclusion of predator interactions or spatial pattern in the current models.

The northern squawfish model of feeding behavior should be helpful in exploring some predation management alternatives. Northern squawfish predation appears to cause high mortality of migrating juvenile salmonids in the Columbia River (Uremovich et al. 1980; Gray and Rondorf 1986; Rieman et al. 1991), prompting recent interest in predation control. Models have been recognized as one way to evaluate predation management actions (Fickeisen et al. 1989). Some management alternatives that could be explored with our simulation model are (1) swamping of the predation response near dams by regulating the diel density of juvenile salmonids, (2) modification of the diel feeding pattern by light changes near a dam, and (3) removal of a portion of the local northern squawfish population. Simulation and interpretation of these alternatives were beyond the scope of this paper but they will be explored in future studies.

From a general predation perspective, models of individual feeding behavior may be useful in estimating some components

in foraging analyses, such as the average duration of feeding bouts, the mean number of captures per bout, and the total time spent foraging during a day (Schoener 1971; Caraco 1980; Lucas 1983; Winterhalder 1983). Sibley et al. (1990) showed how specific models of the processes generating interevent intervals (gaps) can be fit to frequency distributions of gaps, such as intercapture time distributions. Fitted process parameters can be used to compute the mean bout length and the number of bouts, from which total foraging time during a day could be estimated.

Process models could be fit to field or simulated gap distributions; however, the theoretical intercapture time distributions generated by model simulations should be more representative of the true distribution of intercapture times than a distribution derived from gut content data. Predators with zero or one prey in their guts provide no intercapture information. If a predator captures two prey separated by a long period, one or both prey could be completely evacuated by the time the gut is sampled. Thus, long intercapture times would be underrepresented in intercapture frequency distributions from gut samples. Model-generated distributions of intercapture times should be closer to the actual intercapture time distribution than the observed data from predator guts (DeAngelis et al. 1984). Species-specific predation models could provide unbiased descriptions of gap or capture distributions.

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Relationship between Feeding and Activity Rates for Actively Foraging Juvenile Brook Trout (*Salvelinus fontinalis*)

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I tested the hypothesis of the existence of a significant positive linear relationship between activity and consumption rates for an actively foraging fish. Within-day variations in activity rates of 0+ brook trout (*Salvelinus fontinalis*) kept in enclosures were estimated using an underwater videocamera system. Consumption rates at different periods of that day were estimated using the variations of the digestive tract contents. Total consumption rates (i.e. sum of food ingested by all fish) ranged from values close to zero (–1.4 to 0.9; 12:30–24:00) to 12.7 cal/30 min (08:30–09:00). Total activity rates ranged from 0.06 (14:00–14:30) to 2.94 cal/30 min (08:30–09:00). My results indicate that, under specific environmental conditions, activity rates of brook trout are positively related to their feeding rates and, consequently, that the behavior of this actively foraging fish can result in the reduction of the energy losses associated with swimming during nonfeeding periods. My work also permits the development of an experimental protocol to test hypotheses regarding energy allocation patterns between growth, consumption, and activity rates on a multiday basis.

J'ai testé l'hypothèse de l'existence d'une relation positive linéaire significative entre les taux d'activité et de consommation pour un poisson se nourrissant de façon active. Les variations intra-jour des taux d'activité d'ombles de fontaine (*Salvelinus fontinalis*) 0+ gardées en enclos ont été estimées à l'aide d'un système de vidéo-caméras sous-marines. Les taux de consommation à différents moments de cette journée ont été estimés à l'aide des variations des contenus du tractus digestif. Les taux de consommation totale (i.e. somme de la nourriture ingérée par tous les poissons) ont varié entre des valeurs proches de zéro (–1,4 à 0,9; 12:30–24:00) et 12,7 cal/30 min (08:30–09:00). Les taux d'activité totale ont varié entre 0,06 (14:00–14:30) et 2,94 cal/30 min (08:30–09:00). J'ai trouvé une relation positive significative entre les taux d'activité et de consommation. Mes résultats indiquent que dans des conditions environnementales spécifiques les taux d'activité des ombles de fontaine sont positivement reliés aux taux de consommation, et par conséquent, que le comportement de ce poisson résulte en une réduction des pertes énergétiques reliées à la nage durant les périodes lors desquelles il ne se nourrit pas. Mes travaux permettent de développer un protocole expérimental pour tester des hypothèses concernant les patrons d'allocation de l'énergie entre la consommation, la croissance, et l'activité pour plusieurs jours.

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Bioenergetics models are based on the assumption that fish constitute thermodynamically closed systems in which surplus energy (growth plus gonad production; Ware 1982) represents a trade-off between energy intake and expenditures (Winberg 1956; Kitchell et al. 1977; Hewett and Johnson 1987). It has long been recognized that the expression of this trade-off (i.e. fraction of energy intake expressed as surplus energy) may vary with environmental conditions (Paloheimo and Dickie 1966; Pianka 1981; Ricklefs 1991). Evaluation of the influence of specific environmental characteristics on energy allocation patterns is critical to the utilization of bioenergetics models for in situ predictions of fish performance. However, the absolute or relative importance of community characteristics such as fish abundance and specific composition, prey biomass and size, and habitat structural complexity to energy allocation patterns is not known. Adequate accounting for the influence of these environmental conditions on energy allocation patterns is complicated because they are expected to affect two important components of fish energy budget, energy intake and activity costs (Kerr 1971; Ware 1975; Boisclair and Leggett

1989). Unfortunately, these two components are laborious to estimate, particularly in the field.

Kerr (1982, p. 374, his equations 8 and 9) developed a bioenergetic s model in which the activity component is present but algebraically replaced by a relationship between activity and consumption rates. While this operation reduced the number of unknowns in the bioenergetics equation and simplified the study of energy allocation patterns, it added a potentially very useful, but untested, assumption. The only field study that has examined the relationship between activity and feeding rates supports the hypothesis of the existence of a positive linear relationship between these variables (Boisclair and Leggett 1989). As emphasized by Boisclair and Leggett, however, their work does not constitute a true test of the relationship proposed by Kerr because their activity rates were derived by difference using feeding and growth rates.

The purpose of this paper is to present the results of the first experiment designed to directly test the hypothesis of the existence of a positive linear relationship between fish activity and feeding rates. While this hypothesis can be tested using differ-

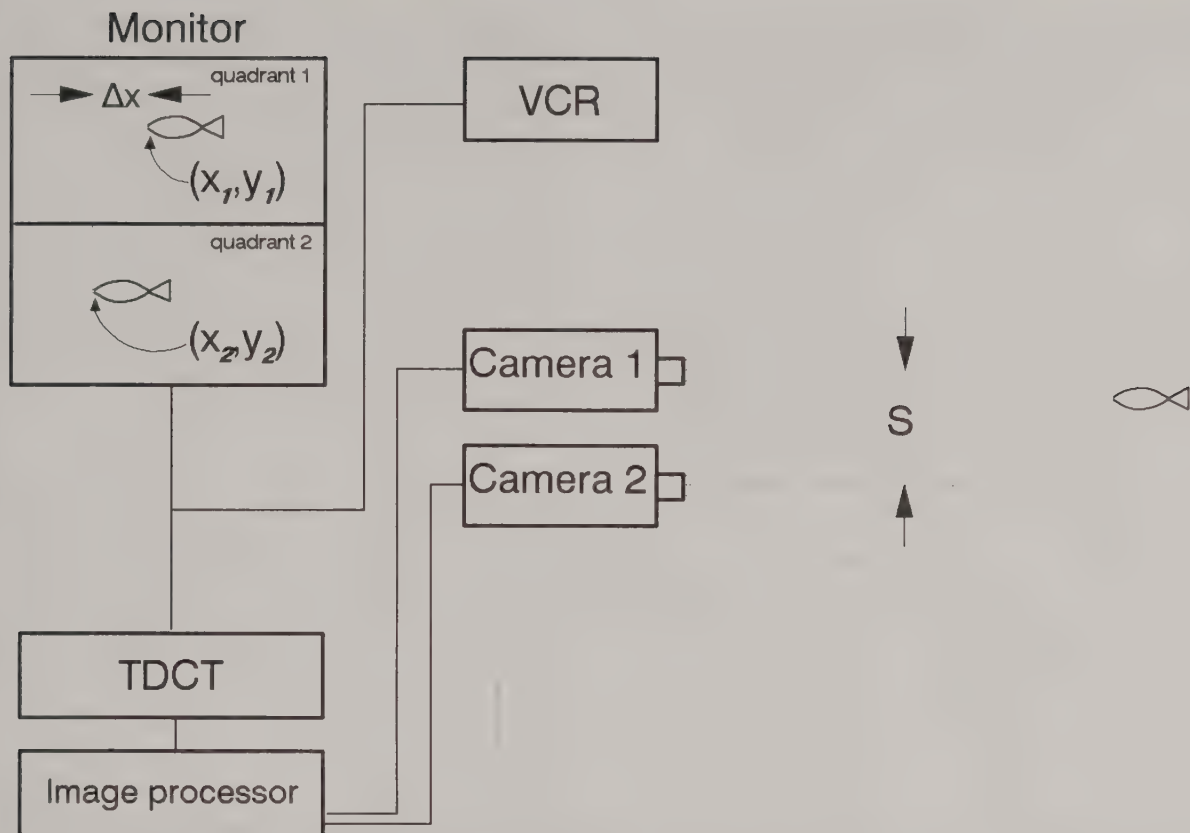


FIG. 1. Schematic representation of the stereocinematographic apparatus. Images perceived by the two cameras are combined and synchronized by an image processor, the camera titler (TDCT) copies the time on the images (hours:minutes:seconds), and these images are recorded using a videocamera recorder (VCR). Characteristics such as optical axes separation (S), coordinates of the targets as they appear in different quadrants of the monitor (x_1, y_1 and x_2, y_2), and the apparent target displacement (Δx) are used to estimate fish coordinates in three dimensions (x, y, z).

ent time scales, the approach taken in this study was to compare feeding and activity rates estimated within 1 d.

Methods

Experimental Approach

Feeding and activity rates were estimated using six 1.5-m³ enclosures (1 × 1 × 1.5 m) submerged and anchored on the 4-m-depth isobath of a mesotrophic lake located at La Station de Biologie des Laurentides de l'Université de Montréal (70 km north west of Montreal), in the lower Laurentian region of Quebec. The mesh size (0.06 cm²) utilized allowed prey such as zooplankton to enter or exit the enclosures.

On August 15, 1990 (15:00–16:00), SCUBA divers introduced five young-of-the-year (YOY) brook trout (*Salvelinus fontinalis*; mean wet weight = 2.14 g, SD = 0.49) into each enclosure through an opening at one end. A pair of videocameras was mounted inside one enclosure which was utilized exclusively for estimating fish activity. Following the introduction of the fish and the installation of the cameras, 750 L of unfiltered lake water was pumped inside each enclosure to reestablish potentially disturbed natural zooplankton biomass inside the enclosures. Fish feeding and activity rates were estimated on August 16, 1990.

Experimental Procedure

Estimation of feeding rates

Estimation of fish feeding rates requires the description of variations of the quantity of food found in the digestive tract of the fish and the determination of evacuation rate (Eggers 1977; Elliott and Persson 1978; Olson and Mullen 1986; Boisclair and Leggett 1988).

The feeding cycle was described using five of the six enclosures. Enclosures are hereafter referred to by the order in which they were sampled. The digestive tract (stomach plus intestine) contents of the five fish in one enclosure were estimated at 3- to 4-h intervals beginning at 01:30 on August 16, 1990. Each 3- to 4-h interval is hereafter referred to as a 3.5-h interval. The sixth and seventh digestive tract content estimates (August 16, 1990; 19:30 and 24:00) required for my analysis were obtained by restocking enclosures 1 and 2 immediately following their sampling (01:00 and 05:30, respectively). Fish were killed by an overdose of 2-phenoxyethanol. Each fish was measured (± 0.5 mm total length), weighed (± 0.005 g wet), and dissected within 2 h after sampling. Digestive tract contents were weighed individually as wet (± 0.00005 g) and dry weights (± 0.00005 g; 48 h at 60°C).

Evacuation rate was estimated in the laboratory. A total of 40 YOY brook trout (mean weight = 4.71 g wet, SD = 1.68) were placed in a 700-L "Living Stream" temperature-regulated stream tank. Fish were kept at 18°C for 1 wk before evacuation

rate was estimated. This temperature was selected because it corresponded to that experienced by fish in the enclosures. Fish were fed ad libitum once daily with thawed brine shrimp (*Artemia* (Anostraca)). As emphasized by Boisclair and Leggett (1988, 1990), this diet can be assumed to adequately represent that of zooplanktivorous fish. Sampling was initiated 0.5 h after fish were fed. At that time, five fish were selected at random from the holding tank and five additional fish were collected at 4-h intervals. Fish were processed as described for the feeding cycle.

Estimation of activity rates

The testing of the Kerr hypothesis requires the estimation of the quantity of energy spent to execute external movements. The term "activity rates" (or cost) is hereafter used to represent the net cost associated with the execution of such movements during a given time interval, excluding other costs that may occur simultaneously (standard metabolic rate, heat increment, etc.; Kerr 1982; Beamish and Trippel 1990). Estimation of fish activity rates requires the determination of swimming speed and its transformation into swimming costs. Swimming speed was estimated using the stereocinematographic method (SCG method; Boisclair 1992). This method is based on the principle that a single target viewed simultaneously by two cameras oriented in parallel in the same direction, and separated by a known distance (i.e. binocular vision), appears at different positions in the two images (Fig. 1). Geometrical algorithms can determine the position of the target in an x, y, z , Cartesian coordinate system. Time series of fish position were used to estimate movements and therefore speed. A complete description of the video apparatus and of the computations utilized in this study was presented by Boisclair (1992). On August 16, 1990, fish were filmed during six 1-h periods: 07:00–09:00, 10:00–11:00, 12:00–13:00, 13:30–14:30, and 18:30–19:30.

Measurements to determine fish position and speed were obtained using the Jandel Video Analysis Software (Java). The position of a target in each image was defined by two pairs of coordinates, one for each quadrant displayed on the monitor used to perform the image analyses (Fig. 1). Each pair of coordinates was determined relative to an origin located in the middle of the quadrant from which it was taken. The x, y, z position of a fish was described by the position of its head and of its tail. The x, y, z position of a fish (head or tail) was determined at 1-s intervals. When more than one fish was visible during a given period, each fish was digitized sequentially until the successive x, y, z positions of all fish observed during that period were estimated.

The transformation of swimming speed into energy expenditure was performed using an empirical relationship between the net cost associated with swimming (expressed as oxygen consumption), fish weight, and routine swimming speed (see Computations).

Estimation of swimming characteristics during feeding and nonfeeding periods

The empirical relationship I used to transform swimming speed into energy expenditure was developed using laboratory experiments performed with unfed fish (Boisclair and Tang 1992). Energetic cost of swimming can be expected to increase with the complexity of the swimming pattern (Smit 1965; Weatherley et al. 1982; Webb 1991; Boisclair and Tang 1992). The transformation I used may therefore underestimate true swimming costs if fish presented with food (as in my enclosures) adopt a more complex swimming pattern than unfed fish. I evaluated the relevance of this bias by testing the hypothesis

that the complexity of fish swimming patterns increased during periods of intense feeding. Swimming characteristics during feeding periods of different intensities were compared using speed, acceleration rates, and angles of turns.

Estimation of zooplankton biomass inside the enclosures

Zooplankton biomass inside the enclosures was estimated on three occasions: in the enclosure containing the videocameras (07:30) and in enclosures 4 and 5 at, respectively, 11:00 and 14:00. This procedure was adopted to minimize the stress of fish recently introduced in enclosures 6 and 7. On each occasion, ten 10-L samples were collected from the enclosures using a battery-powered bilge pump (2800 L/h). Zooplankton was killed by immersion in carbonated water immediately following sampling. Zooplankton weight (± 0.0001 g dry) was determined following filtration on preweighed (± 0.00005 g dry) 1- μ m fiberglass filters and drying at 60°C for 1 h.

Computations

The method I used does not allow the estimation of individual consumption or activity rates. The estimation of digestive tract content requires that fish are killed. Consequently, a given fish cannot be sampled more than once. Furthermore, the limited volume sampled by the videocameras does not permit continuous observation of single fish. Finally, the images recorded do not allow for identification of individual fish. I circumvented these difficulties by estimating the sum of the quantity of energy consumed by the five fish inside the enclosures and the sum of the energy spent swimming by these fish.

Estimation of feeding rates

The quantity of energy consumed by five fish inside the enclosures during each of the six 3.5-h intervals (E_c , calories (1 cal = 4.1868 J)) was estimated as

$$(1) \quad E_c = C_{\Delta t} \cdot B \cdot 5000$$

where $C_{\Delta t}$ is the quantity of food consumed (grams dry weight per 100 g wet weight) by fish during a given 3.5-h time interval, B is the total fish biomass inside the enclosure containing the cameras (grams wet weight), and 5000 represents the average caloric density of zooplankton (calories per gram dry weight; Cummins and Wuycheck 1971).

$C_{\Delta t}$ was estimated using the Elliott and Persson (1978) model:

$$(2) \quad C_{\Delta t} = \frac{(\bar{F}_{t+T} - \bar{F}_t \cdot e^{-RT}) \cdot RT}{(1 - e^{-RT})}$$

where $\bar{F}_t$ and $\bar{F}_{t+T}$ (grams dry weight per 100 g wet weight) are, respectively, the geometric mean weight of the total digestive tract content of fish at the beginning and at the end of a given time interval, T is the length of a time interval (approximately 3.5 h) and R is the evacuation rate (per hour).

$\bar{F}$ was calculated as

$$(3) \quad \bar{F} = \exp \sum_{i=1}^5 \ln (F_i / W_i) \cdot 100$$

where F_i is the individual dry weight (grams) of the total digestive tract content and W_i is the wet weight (grams) of the body of fish i minus the weight of the digestive tract contents.

R was estimated under laboratory conditions as the slope of the relationship between the natural logarithm of F and time since last feeding.

Daily energy intake was estimated as the sum of all feeding rates determined between 01:30 and 24:00. This procedure assumed that the quantity of food consumed by fish in the

1.5-h interval between 24:00 and 01:30 was negligible. This assumption was supported by my data; consumption rates estimated for that time period were slightly negative and not significantly different from 0. Energy intake per 30-min period was derived by dividing each energy consumption rate per 3.5-h interval by 6–8 depending on the number of 30-min periods in the time interval considered.

Estimation of activity rates

The quantity of energy spent swimming by the five fish inside the enclosure was estimated by extrapolating the activity in the portion of the enclosure under observation to the whole enclosure. Total activity cost during a given 30-min period (E_s , calories per 30 min) was estimated as

$$(4) \quad E_s = \frac{A}{pO \cdot pU}$$

where pO is the proportion of the enclosure under observation, pU is the proportion of fish-seconds observed that were effectively used to estimate swimming speed, and A (calories per 30 min) is the energy spent swimming by the fish observed and utilized for computations.

The proportion of the enclosure under observation (0.23) was estimated as the ratio of the volume sampled by the videocamera system (0.346 m³) to the total volume of the enclosure (1.5 m³).

The number of fish-seconds was estimated as the sum of the number of seconds for which at least one fish was observed. When more than one fish was visible during a given second, the number of fish-seconds for that time was equal to the number of fish observed during that second. pU was estimated as the ratio of the number of fish-seconds effectively used to estimate fish swimming speed to the total number of fish-seconds observed. This correction was applied because, while almost all fish filmed were spending some energy inside the observed portion of the enclosure, not all fish observed were effectively utilized to estimate fish swimming speed and cost. The position of a fish was not recorded when it was too close to the lens and out of focus, when it was observed for less than 3 s (the complete fish must be observed for more than 2 s to estimate its speed and acceleration), and when its orientation did not allow discrimination of its head from its tail (estimation of the speed

and angle of turn requires the positioning of the head and of the tail). I observed a total of 1894 fish-seconds and utilized 663 fish-seconds to describe fish swimming speed. pU was therefore 0.35.

A was estimated as

$$(5) \quad A = \sum_{n=1}^N O \cdot 3.24 \cdot 0.000277$$

where N is the number of swimming speed estimates during a given 30-min period, O is the respiration associated with a given swimming speed (milligrams O₂ per hour), 3.24 is an oxyca-
loric equivalent (calories per milligram O₂) allowing the trans-
formation of O₂ consumption into calories, and 0.000277 is the
number of hours in a second.

O was estimated using an empirical relationship between O₂ consumption, fish net body weight (W , grams), and routine swimming speed (V_r , centimetres per second; Boisclair and Tang 1992):

$$(6) \quad \log_{10} O = 0.54 \cdot \log_{10} W + 1.09 \cdot \log_{10} V_r - 0.93.$$

In this study, W was replaced by the mean weight of the fish inside the enclosure containing the videocameras.

V_r was defined as the average speed of the head and of the tail of a fish during a given time interval (1 s). V_r was estimated using the algorithm presented by Boisclair (1992).

Estimation of activity rates during a given 3.5-h interval was based on the interpolation and summation of successive values of activity rates per 30 min (E_s). Daily activity rates were calculated as the area under the curve between activity rates per 30 min and time of day. This calculation was performed using two approaches. First, I assumed that activity rates between dusk and dawn (20:00 and 05:00, respectively) were equivalent to those estimated between 19:00 and 19:30. Second, I assumed that activity rates between dusk and dawn were negligible.

Estimation of swimming characteristics during feeding and nonfeeding periods

Acceleration rates were estimated as the difference in swimming speed of a given fish between two successive time intervals divided by the length of that time interval (generally 1 s). Angle of turn between two consecutive seconds (D_t , degrees) was estimated as

$$(7) \quad D_t = \arccos \frac{[(\bar{x}T_t - \bar{x}Q_t) \cdot (\bar{x}T_{t+1} - \bar{x}Q_{t+1})] + [(\bar{y}T_t - \bar{y}Q_t) \cdot (\bar{y}T_{t+1} - \bar{y}Q_{t+1})] + [(\bar{z}T_t - \bar{z}Q_t) \cdot (\bar{z}T_{t+1} - \bar{z}Q_{t+1})]}{[\sqrt{(\bar{x}T_t - \bar{x}Q_t)^2 + (\bar{y}T_t - \bar{y}Q_t)^2 + (\bar{z}T_t - \bar{z}Q_t)^2}] \cdot [\sqrt{(\bar{x}T_{t+1} - \bar{x}Q_{t+1})^2 + (\bar{y}T_{t+1} - \bar{y}Q_{t+1})^2 + (\bar{z}T_{t+1} - \bar{z}Q_{t+1})^2}]}$$

where $\bar{x}T$, $\bar{y}T$, and $\bar{z}T$ are, respectively the z , y , and x coordinates of the head of the fish at time t or $t + 1$ and $\bar{x}Q$, $\bar{y}Q$, $\bar{z}Q$ are the corresponding coordinates for the tail of the fish. The coordinates of the head or tail of fish at any given time were estimated following the algorithm presented by Boisclair (1992).

Data Analysis

I used one-way analysis of variance to examine variations of the digestive tract content of fish collected at different times of day and variations of the zooplankton biomass among the three enclosures surveyed. The relationship between feeding and activity rates per 30-min or 3.5-h intervals was tested using regression analysis. Swimming speed, acceleration, and angle of turn between feeding and nonfeeding periods were compared following three steps. First, each value of a given swimming

characteristic was ranked using the Blom procedure (Blom 1958). Second, the ranks were normalized using the ψ transformation (Conover and Iman 1981). Third, the swimming characteristics were compared using parametric one-way analysis of variance on the ranked normalized values.

Results

Estimation of Feeding Rates

Mean quantity of food found in the digestive tract of fish ranged from 0.07 (01:30) to 0.36 g dry/100 g wet (12:30) (Fig. 2a). These values varied significantly among enclosures sampled at different times of the day ($F_{6,28} = 9.50$; $p < 0.0001$). Evacuation rate estimated under laboratory conditions, ($F_{1,33} = 69.54$; $p < 0.0001$; $r^2 = 0.66$) was 0.075/h (SE = 0.009). Mean food consumption rates per 3.5-h interval ranged

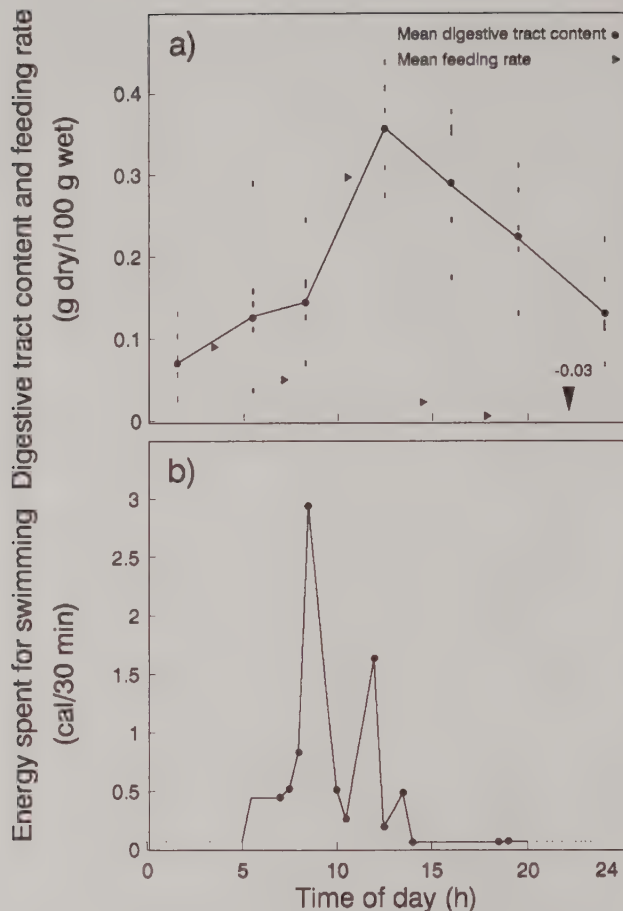


FIG. 2. (a) Variations in the digestive tract contents and feeding rates at different times of the day. Marks and circles represent individual and geometric mean values of digestive tract contents. Feeding rates per 3.5-h interval are represented by triangles positioned in the middle of each time interval. The negative feeding rate obtained between 19:30 and 24:00 is not represented. (b) Total energy spent swimming by the five fish observed during the twelve 30-min periods recorded. Circles and solid lines represent estimated and interpolated values, respectively. Dotted lines represent the swimming costs if activity rates during the night are equal to those estimated at dusk (19:00–19:30).

from values close to zero (-0.03 to 0.0017 ; 12:30–24:00) to 0.29 g dry/100 g wet $^{-1}$ (08:30–12:30). Maximum quantity of energy consumed by five fish during a 3.5-h interval (maximum E_c) was 105 cal (08:30–12:30). Corresponding energy consumption rate per 30 min was 12.7 cal.

Daily ration was 0.41 g dry/100 g wet. Total quantity of energy consumed by five fish during the day was 147.2 cal. Fish consumed 83% of their daily energy intake between 05:30 and 12:30. The 05:30–12:30 and 12:30–19:30 intervals are hereafter referred to as the “feeding” and “nonfeeding” periods, respectively.

Estimation of Activity Rates

Swimming speed ranged from 0.4 to 39.1 cm/s. However, 86% of the speed estimates were lower than 10 cm/s. Average swimming speed during specific 30-min periods ranged from 2.8 (08:00–08:30) to 8.4 cm/s (12:00–12:30). Speed varied sig-

nificantly among 30-min periods ($F_{11,647} = 4.28$; $p < 0.0001$). Energy spent swimming per 30-min periods (sum of the five fish observed) ranged from 0.06 (14:00–14:30) to 2.94 cal (08:30–09:00) (Fig. 2b). Activity costs during specific 3.5-h periods ranged from 0.5 (16:00–19:30) to 10.7 cal (8:30–12:30).

Total quantity of energy spent swimming by five fish during the day was 17.1 cal when activity rates between dusk and dawn (20:00 and 05:00, respectively) were assumed equivalent to those estimated between 19:00 and 19:30. This value represented 12% of fish energy budget (i.e. percentage of daily energy intake). Fish spent 81% of their daily activity costs during the feeding period. When activity rates during the night were assumed negligible, daily activity rates were 15.7 cal and represented 11% of the energy budget. Following this assumption, fish spent 88% of their activity costs during the feeding period.

Estimation of Zooplankton Biomass Inside the Enclosures

Mean zooplankton biomass inside the enclosures ranged from 0.044 (14:00; SD = 0.029) to 0.056 mg dry/L (11:00; SD = 0.015). Zooplankton biomass did not differ significantly among the enclosures sampled ($F_{2,27} = 0.37$; $p > 0.60$). The grand mean zooplankton biomass was 0.050 mg dry/L (SD = 0.031).

Relationship between Activity and Feeding Rates

I found a significant positive relationship between activity and feeding rates estimated for the twelve 30-min periods ($F_{1,10} = 16.62$; $p < 0.003$; $r^2 = 0.62$). The best model found was logarithmic. The slope of this relationship (0.54 ; SE = 0.13) was significantly different from unity, indicating that the relationship between activity and feeding rates was not linear (Fig. 3a).

There was a significant positive relationship between the natural logarithm of activity and consumption rates estimated at 3.5-h intervals ($F_{1,2} = 75.74$; $p < 0.02$, $r^2 = 0.97$). The slope of the relationship (0.59 ; SE = 0.07) was significantly different from unity (Fig. 3b).

Comparison of Swimming Characteristics during Feeding and Nonfeeding Periods

Swimming speeds were skewed towards high values (Fig. 4a). Average swimming speeds during feeding and nonfeeding periods were respectively, 6.35 and 5.33 cm/s. During the nonfeeding period, fish never swam faster than 15 cm/s. In contrast, these speeds accounted for 4% of the estimates during the feeding period (Fig. 4a). Swimming speed during the feeding and nonfeeding periods did not vary significantly ($F_{1,656} = 3.34$; $0.07 > p > 0.05$).

Acceleration rates averaged -0.01 and 0.21 cm/s 2 during the feeding and nonfeeding periods, respectively (Fig. 4b). Acceleration rates ranging from -10 (deceleration) to 10 cm/s 2 represented 93.5 and 100% of the values estimated during the feeding and nonfeeding periods, respectively. Acceleration or deceleration rates greater than 10 cm/s 2 represented 6.5% of movements observed during the feeding period. Such rapid changes in swimming speed were never observed during the nonfeeding period (Fig. 4b). However, I found no significant differences between average acceleration rates estimated for the feeding and nonfeeding periods ($F_{1,540} = 0.17$; $p > 0.65$).

Angles of turns during the feeding period ranged from 0 to 167° (average = 35°); corresponding values for the nonfeeding

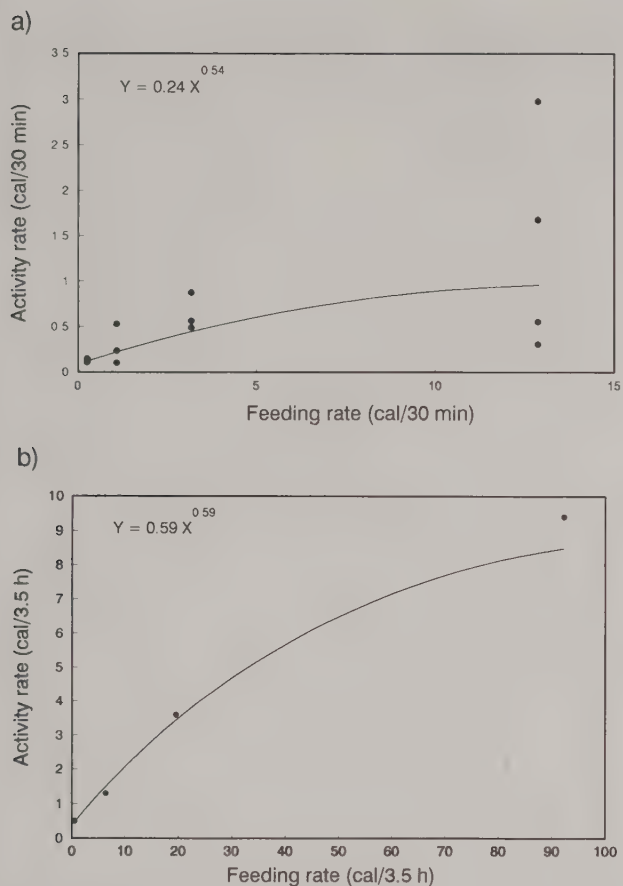


FIG. 3. Relationship between activity and feeding rates estimated on a (a) 30-min and (b) 3.5-h time scale.

period were 0.7 and 114° (average = 28°) (Fig. 4c). Angles of turns ranging from 0 to 50° accounted for 78% of estimated values for both the feeding and nonfeeding periods. Angles of turns larger than 100° represented 8% of the values during the feeding period compared with 5% during the nonfeeding period. I found no significant difference between angles of turns estimated during the feeding and nonfeeding periods ($F_{1,666} = 3.73$; $0.06 > p > 0.05$).

Discussion

My analyses support the hypothesis of the existence of a significant positive relationship between activity and feeding rates within 1 d. This finding suggests that, under relatively simple environmental conditions (constant food biomass, absence of interspecific competitors and predators, low habitat complexity), the behavior of an actively foraging fish can result in the reduction of energy losses associated with swimming during nonfeeding periods. The existence of relationships between activity and feeding rates for fish characterized by different feeding strategies (e.g. ambush predators, drift feeders) is not known and requires further investigation.

The trade-off between consumption, growth, and activity rates has been argued to vary significantly with fish size and reproductive status, prey size, abundance, and distribution (Paloheimo and Dickie 1966; Kerr 1971; Ware 1975; Koch and Wieser 1983; Boisclair and Leggett 1989). Fish characteristics

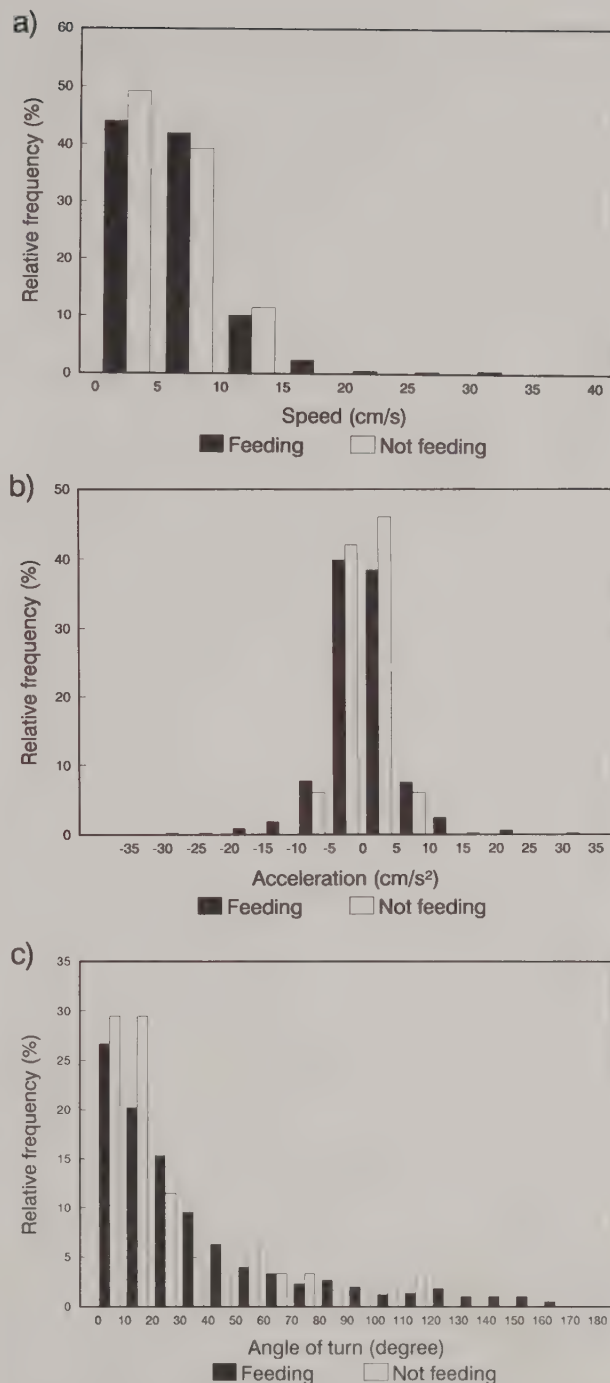


FIG. 4. Frequency distribution of (a) swimming speeds, (b) acceleration rates, and (c) angles of turn during the feeding (07:00–12:30) and nonfeeding (12:30–19:30) periods.

and environmental conditions may therefore affect the shape, equation, or existence of the relationship between activity and feeding rates. While the behavioral regulation of the balance between costs and benefits of an actively foraging fish is consistent with the assumption taken by Kerr (1982) to construct his model, the acceptance of that model and the integration of the relationship between activity and feeding rates to bioener-

getics models are conditional to its testing under a variety of experimental conditions. Consequently, the contribution of this study may not be the specific relationship derived, but the method used and the direct demonstration that such a relationship may exist for an actively foraging fish.

My results contrast with the model suggested by Kerr (1982) by indicating that the relationship between activity and feeding rates is not necessarily linear but that it may be logarithmic with a slope in the 0.5–0.6 range. It is appropriate to examine the two assumptions I used in arriving at this conclusion. The first assumption is that feeding rates derived using two different enclosures can be coupled with activity rates derived from another enclosure. This assumption may be valid when fish number and size as well as prey biomass and water temperature are kept relatively constant among enclosures. Although these conditions were adhered to in my experiment, particularly during the period of intense feeding and activity (07:30–14:00), the variances associated with the relationships I found indicate that a perfect temporal coupling between activity and feeding rates of fish kept in different enclosures may be impossible. This problem is best exemplified by two low activity rates classified as occurring during an intense feeding period (Fig. 3a). This situation may arise because fish behavior differs among enclosures and because fish may not continuously feed at an equal average rate during a period of intense feeding. Under such circumstances, the estimation of feeding rates per 30-min periods using *intrappolation* (i.e. consumption rates on a 30-min period = consumption rates per 4 h divided by 8) may be a valid procedure on average, but may sometimes result in unrealistic activity and feeding rate combinations. Comparisons of activity and feeding rates based on complete 3.5-h intervals are expected to be less subjected to this problem. Such comparisons nevertheless indicate that the relationship between activity and feeding rates is logarithmic. It is important to note, however, that this relationship was based on only four points and that additional data may influence the shape of the relationship between activity and feeding rates. Furthermore, it has been suggested that surveys of variations in fish stomach rather than complete digestive tract contents may permit more precise determination of the timing of feeding (Boisclair and Leggett 1988; D. Boisclair and Marchand, pers. obs.). Consequently, it can be expected that the coupling between feeding and activity rates may be improved by sampling stomach instead of complete digestive tract contents. The second assumption I used was that routine swimming costs are adequately represented by the empirical relationship presented by Boisclair and Tang (1992) (see Computations). This relationship was developed using swimming speed and respiration rates estimated on unfed fish. My results (Fig. 4b and 4c) indicate that fish may adopt slightly more complex swimming patterns from a nonfeeding to a feeding period. While the statistical significance of the difference between swimming patterns observed during feeding and nonfeeding periods is marginal, the physiological implications of these small differences are not known. Because empirical and theoretical models suggest that swimming costs increase with increasing swimming complexity (Smit 1965; Weatherley et al. 1982; Forstner and Wieser 1990; Webb 1991; Boisclair and Tang 1992), it may be realistic to speculate that my estimates of activity costs, particularly during periods of intense feeding, and by extension of my estimate of the proportion of the energy budget allocated to activity (11–12% depending on the assumption used to estimate fish activity costs during the night; see Results), may underestimate true values. Current knowledge about swimming costs associated with

swimming patterns of varying complexity does not allow an accurate estimation of the magnitude of this bias. However, it can be expected that this bias could change the shape of the relationship between activity and feeding rates. Further research in this area appears critical to the accurate estimation of fish activity costs.

Quantitative description of energy allocation patterns and evaluation of the influence of specific environmental characteristics on energy allocation patterns dictate that consumption, growth, and activity rates must be estimated under experimental conditions and temporal scales that allow significant consumption, growth, and activity rates to occur. This condition relative to growth rates was not adhered to in my experiment. The duration of my experiment may also be partly responsible for the difference between the shape of the relationships I obtained (logarithmic) and those found by Kerr (1982) and Boisclair and Leggett (1989) on a temporal scale of weeks to months (linear). However, my work does contribute to the elaboration of an experimental protocol to more fully examine energy allocation patterns. My study suggests that the quantity of food consumed by fish per day can be estimated using a series of enclosures. My work also illustrates that underwater videocameras can be effectively utilized to estimate activity costs of fish kept in enclosures. The most difficult problem associated with the estimation of activity costs under a time scale sufficient for significant growth to occur is the estimation of fish activity during the night. While my results do not allow me to clearly establish the shape of the relationship between activity and feeding rates, my analyses do support the existence of a positive relationship with an intercept close to 0. Consequently, activity rates of 0+ brook trout that do not feed during the night can be expected to be negligible. Finally, growth rates can be determined using the fish kept inside the enclosure used for estimating fish activity rates. It is worth noting that this protocol is not suitable for testing all variables that may affect energy allocation patterns, and consequently, it may not provide results that can be directly extrapolated to field situations. For instance, the presence of piscivores has been shown to affect fish behavior and habitat utilization (Mittelbach 1984) and may be difficult to simulate experimentally. The approach I propose, however, can be expected to enable the description of general trends regarding the influence of specific environmental conditions such as water temperature, O₂ concentration, prey size, and biomass on the partitioning of energy consumed between growth and activity rates.

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Population Genetics of the Hard Clam, *Mercenaria mercenaria*, at the Northern Limit of Its Range¹

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Canadian populations of *Mercenaria* deserve recognition as stocks distinct from the larger population of the U.S. Atlantic coast. Although the hard clam occupies a virtually continuous range from Florida to Massachusetts, its distribution north of Cape Cod becomes disjunct. Here, we use protein electrophoresis to determine gene frequencies at seven polymorphic enzyme loci in clam populations from Maine, USA, and New Brunswick and Prince Edward Island, Canada, and compare our results with previously published data from Massachusetts. The fit to the Hardy–Weinberg expectation within populations was very good. The Maine population showed small but statistically significant divergence from its putative source population to the south at most loci, with an apparent loss of two rare alleles. Both Canadian populations showed larger levels of divergence, with the loss of 6–12 alleles and significant reductions in overall heterozygosity. The recognition of a St. Lawrence stock of hard clams at Prince Edward Island may have important implications for the fishery.

Les populations de *Mercenaria* du Canada devraient être reconnues comme étant des stocks distincts de la population plus importante de la côte atlantique des États-Unis. Bien que la palourde américaine occupe une aire pratiquement continue depuis la Floride jusqu'au Massachusetts, au nord de Cape Cod, sa répartition devient discontinue. Dans la présente étude, nous avons utilisé l'électrophorèse des protéines pour déterminer la fréquence génétique sur sept loci à enzyme polymorphes chez les populations de palourdes américaines du Maine (É.-U.) ainsi que du Nouveau-Brunswick et de l'Île-du-Prince-Édouard (Canada); nous avons ensuite comparé nos résultats à des données du Massachusetts publiées antérieurement. Les résultats correspondaient très étroitement au modèle prévu en fonction de la loi de Hardy–Weinberg. La population du Maine présentait une divergence légère, mais importante sur le plan statistique, sur la plupart des loci, par rapport à sa population d'origine présumée située plus au sud, conjuguée à la perte apparente de deux allèles rares. Dans les deux populations canadiennes, outre la perte de 6 à 12 allèles et des diminutions dans la proportion globale d'hétérozygotes, le degré de divergence était plus élevé. La reconnaissance d'un stock de palourdes américaines du Saint-Laurent, à l'Île-du-Prince-Édouard, pourrait avoir une incidence considérable sur la pêche à ce mollusque.

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M*ercenaria mercenaria* (L.), the commercial hard clam or quahog, is a common inhabitant of the U.S. Atlantic coast from Florida to Massachusetts (Menzel 1989). The dispersal capabilities of its larvae seem to be sufficient to maintain virtual panmixia over its entire range. Comparing *Mercenaria* populations from Massachusetts and Virginia, Dillon and Manzi (1987) detected a significant difference in allele frequency at only one of seven polymorphic enzyme loci. Similarly low levels of divergence have been reported among Virginia, South Carolina, and Florida populations (Dillon and Manzi 1989; Dillon 1992a).

Between Cape Cod and the Gulf of St. Lawrence (Fig. 1), however, *Mercenaria* populations have a patchy distribution. In Maine, they are reliably collected only in shallow estuaries warmed by the sun or by addition of freshwater. During the period 1939–67 only three substantial recruitment events

occurred in the Gulf of Maine (1937, 1947, and 1952), all associated with unusually high sea temperatures (Dow 1973). Natural *Mercenaria* populations are even more rare on the eastern Coast of New Brunswick and Nova Scotia. Although Whiteaves (1901) quoted secondary reports of hard clams at St. Mary's Bay on the Nova Scotian side of the Bay of Fundy, and on the Atlantic coast of Nova Scotia, shellfish biologists with the Nova Scotia Department of Fisheries report no knowledge of natural *Mercenaria* populations in this region. No Atlantic or Bay of Fundy populations were mentioned by Bourne (1989) in his review of Canadian clam fisheries.

We surveyed the collections of five major museums: the U.S. National Museum in Washington, the Academy of Natural Sciences in Philadelphia, the American Museum of Natural History in New York (AMNH), the Museum of Comparative Zoology at Harvard (MCZ), and the Canadian Museum of Nature in Ottawa. Both the AMNH and MCZ have specimens of *Mercenaria* from the area of St. Andrews, New Brunswick. However, the museums contain no records of the species from the Bay of Fundy, and only a single sample (at the Canadian Museum) from the Atlantic coast of Nova Scotia near Halifax.

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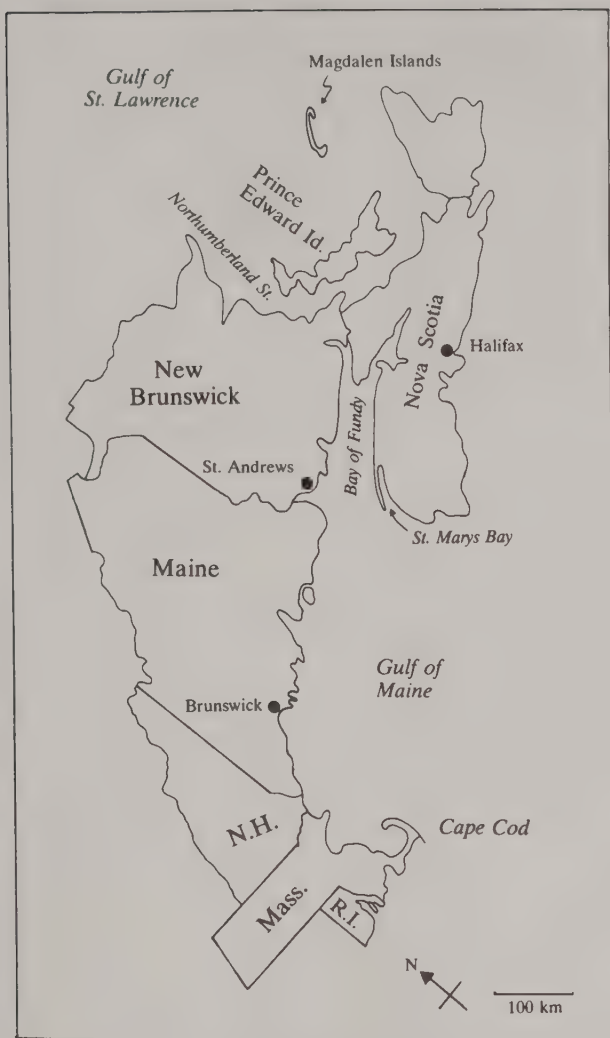


FIG. 1. United States and Canada at the northern limit of the range of *M. mercenaria*. R.I., Rhode Island; Mass., Massachusetts; N.H., New Hampshire. Populations of clams sampled were from Prince Edward Island and sites near St. Andrews, New Brunswick, and Brunswick, Maine.

Bathed by the warmer waters of the St. Lawrence River, *Mercenaria* populations again reach commercially harvestable levels in the Northumberland Strait on the northern coasts of New Brunswick and Nova Scotia, as well as Prince Edward Island (P.E.I.) (Bourne 1989). The northern limit of *Mercenaria*'s range is generally considered to be at the Magdalen Islands, in the Gulf of St. Lawrence. Given the virtual isolation of the St. Lawrence populations, it would seem possible that they constitute a stock genetically distinct from that inhabiting the Atlantic coast of the United States.

Protein electrophoresis has often proven to be a useful tool for the identification and characterization of fish stocks. A search of the Aquatic Sciences and Fisheries Abstracts for the years 1982–91 yielded 114 titles with both "stock identification" and "electrophoresis" among the descriptors. The topic has been thoroughly reviewed in the proceedings of several international meetings (Berst and Simon 1981; Kumpf 1987; Ryman and Utter 1987). Isozyme data do not seem to suggest

the existence of natural stocks of edible mussels (Koehn 1991), although population differentiation is occasionally noted in scallops (Beaumont 1991) and oysters (Fujio 1979; Buroker 1983).

To date, the only data bearing on the question of a northern *Mercenaria* stock are those gathered by Pesch (1972, 1974). His sample of 50 clams from P.E.I. showed gene frequencies strikingly different from those of Maine, Rhode Island, or South Carolina at two loci: one encoding lactate dehydrogenase and the other superoxide dismutase ("TO"). He noted much less divergence at two other, weakly polymorphic loci encoding malate dehydrogenase and an esterase. In this investigation, we used larger sample sizes and a larger number of loci to examine in greater detail the levels of genetic divergence at the northern extreme of *Mercenaria*'s natural range.

Materials and Methods

Populations of *M. mercenaria* sampled for this study were from an intertidal site near the city of Brunswick, Maine, from Sam Orr's Pond about 10 km east of St. Andrews in the province of New Brunswick, and from P.E.I. (Fig. 1). The environment of Sam Orr's Pond is especially unusual. The pond receives both a fairly constant freshwater input from a small stream and a tidal input of seawater from the Bay of Fundy. During the winter the freshwater freezes over the seawater, protecting the *Mercenaria* population beneath from more severe temperatures.

Samples were obtained by raking and hand-picking and include only the largest size classes available. Standard lengths of shells ranged from 8.5 to 11.5 cm in the Maine sample, from 5.4 to 10.0 cm in Sam Orr's Pond, and from 7.9 to 10.3 cm at P.E.I. Voucher specimens from all three populations have been deposited in the collections of both the Canadian Museum of Nature and the Academy of Natural Science of Philadelphia.

We compared allele frequencies at seven enzyme loci in these populations with data previously published on *Mercenaria* from Massachusetts (Dillon and Manzi 1987). The electrophoretic techniques employed here have been described previously (Dillon 1982, 1985). We examined isozyme variation in 6-phosphogluconate dehydrogenase (*Pgd*), glucose-6-phosphate isomerase (*Gpi*), superoxide dismutase (*Sod*) two phosphoglucomutase loci (*PgmF* and *PgmS*), mannose-6-phosphate isomerase (*Mpi*), and leucine aminopeptidase (*Lap*). Multiple buffer systems were used to resolve several of these loci. The first two loci were examined using an aminopropylmorpholine pH 6 buffer, the second, third, and fourth loci were resolved using the Poulik discontinuous buffer system, and for the last four loci, a Tris – citric Acid pH 6 buffer was used. A detailed description of all our electrophoretic equipment, materials, and methods is given in Dillon (1992b). Mendelian inheritance of isozyme phenotype at five of these loci, *Pgd*, *Gpi*, *PgmF*, *PgmS*, and *Lap*, has been verified by Adamkewicz et al. (1984).

We used BIOSYS-1 (Swofford and Selander 1981) to calculate gene frequencies, heterozygosities, Wright's *F*-statistics (Wright 1978), and Nei's unbiased genetic distance (Nei 1978). Two separate analyses of population differentiation using *F*-statistics were performed. In the more conventional analysis, mean F_{is} , F_{it} , and F_{st} over seven loci were calculated within and among the four populations: Massachusetts, Maine, Sam Orr's Pond, and P.E.I. We also performed a hierarchical analysis, grouping the two U.S. populations and comparing this group with the two separate Canadian populations. Mean *F*-statistics calculated in this fashion were labelled F_{ch} (population

TABLE 1. Samples sizes (N), allele frequencies, and observed heterozygosities (H) at seven loci in four samples of *M. mercenaria* (data on the Massachusetts population taken from Dillon and Manzi 1987).

Locus	Allele	Massachusetts	Maine	Orr's	P.E.I.
<i>Gpi</i>	N	79	123	156	199
	110	0.006	0.004	0.010	0.005
	105	0.019	0.004	0.000	0.018
	100	0.848	0.882	0.990	0.952
	90	0.019	0.024	0.000	0.000
	80	0.006	0.057	0.000	0.000
	70	0.063	0.028	0.000	0.025
	60	0.038	0.000	0.000	0.000
	H	0.278	0.228	0.019	0.095
<i>Mpi</i>	N	65	122	131	183
	108	0.038	0.008	0.008	0.046
	105	0.307	0.488	0.676	0.533
	100	0.354	0.430	0.286	0.235
	95	0.281	0.074	0.031	0.186
	H	0.600	0.533	0.450	0.617
<i>Lap</i>	N	77	123	156	191
	104	0.084	0.093	0.000	0.131
	100	0.448	0.411	0.580	0.542
	96	0.448	0.476	0.375	0.319
	94	0.019	0.020	0.045	0.008
	H	0.623	0.650	0.494	0.613
<i>Sod</i>	N	78	123	109	179
	100	0.692	0.533	0.339	0.338
	90	0.308	0.467	0.661	0.662
	H	0.410	0.447	0.422	0.486
<i>Pgd</i>	N	78	123	118	180
	110	0.006	0.053	0.000	0.050
	100	0.647	0.553	0.606	0.625
	90	0.346	0.382	0.394	0.325
	80	0.000	0.012	0.000	0.000
	H	0.602	0.642	0.398	0.489
<i>PgmS</i>	N	78	122	118	185
	103	0.019	0.016	0.064	0.019
	100	0.731	0.693	0.818	0.773
	97	0.027	0.053	0.119	0.065
	92	0.154	0.123	0.000	0.059
	87	0.071	0.115	0.000	0.084
	H	0.449	0.484	0.322	0.384
<i>PgmF</i>	N	77	122	149	198
	105	0.040	0.000	0.000	0.000
	103	0.104	0.102	0.483	0.008
	100	0.857	0.877	0.517	0.992
	97	0.000	0.020	0.000	0.000
	H	0.260	0.230	0.376	0.015

to "hierarchy"), F_{HT} , and F_{ST} . The fit to Hardy-Weinberg equilibrium within each population was tested using goodness-of-fit χ^2 tests, combining rare genotypic classes as necessary. The overall heterozygosity of each population was compared with all others by means of χ^2 contingency tests, corrected for continuity, totalling the number of heterozygous genotypes observed over all individuals and all loci.

Populations were compared by their gene frequencies at individual loci using χ^2 contingency tests, corrected for continuity in 2×2 cases, again combining rare alleles as required. Exact binomial tests were used to assess the significance of the apparent absence or low frequency of individual alleles from particular populations. For example, if the frequency of allele *PgmF* 105 in Massachusetts is fairly estimated at 0.040, the chance that its absence from 122 Maine clams is due to sampling error

would be $(1 - 0.040)^{244} = 5 \times 10^{-5}$. In applying this method, we take 0.040 as a parameter, rather than the estimate that it is. Thus the probability obtained (5×10^{-5} in this example) is also only an estimate, but nonetheless often highly suggestive of significance.

Results

Gene frequencies at seven enzyme loci from the three northern populations are given in Table 1, along with previously published results from Massachusetts. Fits to Hardy-Weinberg equilibrium within populations were generally very good. Since $3 \times 7 = 21$ loci were newly examined for this study, Bonferroni correction would suggest a value of χ^2 nominally significant at the $p = 0.05/21 = 0.0024$ level to reject Hardy-

TABLE 2. Above the diagonal are values of χ^2 comparing overall observed heterozygosities between all pairs of four northern *Mercenaria* populations. Values greater than 3.84 are significant at or above the 0.05 level. Below the diagonal are Nei's (1978) unbiased genetic distances.

	Massachusetts	Maine	Orr's	P.E.I.
Massachusetts	—	0.00	15.6	8.33
Maine	0.012	—	20.0	11.1
Sam Orr's Pond	0.085	0.066	—	2.26
P.E.I.	0.042	0.026	0.066	—

Weinberg (Rice 1989). No value of χ^2 this extreme was in fact observed. Mean heterozygosities over all seven loci, by direct count, were 0.460, 0.459, 0.354, and 0.386 for Massachusetts, Maine, Sam Orr's Pond, and P.E.I. respectively. These figures agree well with their respective Hardy-Weinberg expected heterozygosities: 0.442, 0.450, 0.393, and 0.380. Table 2 shows that the two U.S. populations did not differ significantly in overall observed heterozygosity, nor did the two Canadian populations. The two Canadian populations were, however, both significantly less heterozygous than the two U.S. populations.

Although not striking, most of the allele frequency differences between Massachusetts and Maine *Mercenaria* populations (Table 1) are significant at the 0.05 level. Chi-square contingency tests showed significant differences between the two U.S. populations at the *Mpi* and *Sod* loci. The unexpectedly high frequency of *Pgd 110* in Maine was significant by an exact test, as were the absences of *Gpi 60* and *PgmF 105*. Note, however, that *PgmF 97* and *Pgd 80* were present in Maine but apparently missed in Massachusetts. (These alleles have been observed further south, and their absences from the smaller Massachusetts sample are not significant). Thus, there was no net difference between the Massachusetts and Maine populations of *Mercenaria* in number of alleles observed.

The two Canadian populations were significantly different from the two U.S. populations in allele frequencies at all loci examined. Interestingly, the U.S. populations were more similar to the P.E.I. population than to the geographically closer Sam Orr's Pond population. The Maine and Massachusetts populations were the most genetically similar pair of populations, followed by the Maine/P.E.I. pair (Table 2). In addition to missing the two alleles previously noted absent from Massachusetts and the two absent from Maine, the P.E.I. population was significantly missing two other GPI alleles, *Gpi 90* at 0.024 in Maine and *Gpi 80* at 0.057. The Sam Orr's Pond population was missing these six alleles as well as six others: *Lap 104*, *Pgd 110*, *PgmS 92*, *PgmS 87*, *Gpi 70*, and *Gpi 105*, all but the last significant, using Maine frequencies as expected. Table 1 shows several other striking genetic features of the Canadian populations, including the reversal in relative frequencies of the two *Sod* alleles and the very high frequency of *PgmF 103* in Sam Orr's Pond.

The value of F_{is} (Table 3) differed from 0.0 only by rounding error, as may be expected when genotype frequencies match Hardy-Weinberg expectation within populations. Then, $F_{st} = F_{it}$, and all the gene diversity is attributable to differentiation between populations. The observation that F_{ht} accounts for most of the total F_{st} confirms that divergence is due primarily to differentiation by the two Canadian populations from the larger U.S. population. In Wright's island model, a value of $F_{st} = 0.05$ implies that Nm , the average number of individuals moving among populations, equals 4.75 (Slatkin and Barton 1989). This suggests that although these populations are distinct, gene flow has been strong enough to prevent substantial differentiation among them due to genetic drift.

TABLE 3. Wright's F -statistics (averaged over seven loci) measuring the amount of differentiation between individuals (i), population (s), hierarchy (h, defined in the text), and total (t).

Comparison	Coefficient
F_{is}	-0.001
F_{st}	0.050
F_{it}	0.049
F_{sh}	0.013
F_{ht}	0.034
F_{st}	0.047

Discussion

Although commercially important sets of hard clams occur infrequently in Maine, local densities may be quite great. Dow and Wallace (1965) reported densities as high as 25 000 clams·ft⁻² (270 000 clams·m⁻²) shortly after the 1947 set. We speculate, therefore, that the northern migration of *M. mercenaria* may have occurred episodically, i.e. that exceptional years of reproduction and dispersal may have been followed by longer periods of high mortality in all but a few isolated refugia. The apparent loss of rare alleles from all three clam populations newly examined here is compelling evidence of such bottlenecking.

Ihssen et al. (1981) have defined a fisheries stock as "an intraspecific group of randomly mating individuals with temporal or spatial integrity". None of the populations examined here could be considered randomly mating with any other. But although significant, the genetic differences apparent between the Maine and Massachusetts populations are of the order of magnitude we have previously ascribed to isolation by distance (Dillon and Manzi 1987, 1989). We can identify no obvious barrier to gene flow between Massachusetts and Maine. Thus, in the case of the Maine population, the evidence for "temporal or spatial integrity" is not strong.

By contrast, the divergence of the Sam Orr's Pond and P.E.I. populations, both from more southern populations and from each other, is much more striking. Our results confirm and expand upon those of Pesch (1974), suggesting both that Canadian populations of *Mercenaria* are genetically distinct from their putative source population to the south and that they show reduced variation. Taken together with the observed distribution of *Mercenaria* in Canada, the data of Tables 1 and 2 suggest strongly that these be considered distinct stocks.

The Sam Orr's Pond population is small and not important commercially, although it supports occasional recreational use. Its origin and age are unknown; *Mercenaria* have been taken from the pond as long as anyone can remember. Our findings here are interesting as a confirmation of population genetics theory, which predicts that small populations may initially be characterized by reduced allelic diversity (Leberg 1992).

The recognition that the *Mercenaria* of the Gulf of St. Lawrence and the Northumberland Strait comprise a distinct stock has management implications for the Canadian hard clam fishery. Bourne (1989) reported that Canadian landings of *Mercenaria* between 1951 and 1981 have fluctuated over an order of magnitude, from 2040 to under 200 t annually. He attributed this variability to localized distribution of stocks, environmental degradation, and overexploitation. It is clear that the unusually slow growth rates observed in Canadian *Mercenaria* (6–7 yr to market size) contribute to the danger of overfishing, as does the correspondence between optimum harvest size and age at first reproduction. The evidence presented here that little annual immigration from the vast U.S. population is to be expected may be cause for additional concern.

There have also been a number of experiments with hard clam mariculture, both on P.E.I. and on the northern coast of New Brunswick (Bourne 1989). Given the relatively low genetic variability of the St. Lawrence stock, some effort at outbreeding with U.S. stock, along the lines pursued by Manzi et al. (1991), may be desirable.

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Periphyton Response in an Industrial Receiving Stream: Lipid-based Physiological Stress Analysis and Pattern Recognition of Microbial Community Structure

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Periphyton were collected from three physically similar sites on an industrial receiving stream which differed in their distances from the discharge. Previous studies had established that biological effects diminished with distance downstream from the discharges. In this study, a multivariate analysis of periphyton membrane lipid profiles quantitatively verified site-specific differences in time-independent microbial community structure qualitatively suggested by algal taxonomic analyses. There were no differences in periphyton abundance among sites. Periphyton physiology was evaluated using a ¹⁴C-labeled bicarbonate and ³H-labeled amino acids dual-label incubation, with subsequent analysis of carbon partitioning into lipid compartments. Total radiolabel uptake was similar for all sites. Physiological stress was shown to be highest at the site closest to the discharges using a membrane to storage lipid synthetic ratio. High values of this ratio indicated that both the phototrophic and heterotrophic constituents were stressed, and this stress declined with distance downstream. The ability to measure algal and bacterial abundance, community structure, activity, and physiological status from a single extraction provides a powerful method to evaluate periphyton which can serve as a useful biomonitoring and toxicity assessment tool for aquatic ecosystems.

Dans un cours d'eau récepteur d'effluents industriels, nous avons prélevé du périphyton à trois endroits physiquement similaires mais situés à différentes distances du point de rejet. Des études antérieures avaient établi que les effets biologiques diminuaient proportionnellement à l'augmentation de la distance en aval par rapport aux points de rejet. Dans la présente étude sur le périphyton, une analyse multidimensionnelle des profils lipidiques de la membrane cellulaire confirme quantitativement les différences particulières aux sites dans la structure de la communauté microbienne, indépendamment du temps; l'hypothèse relatives à ces différences était issue d'analyses taxinomiques qualitatives des algues. Nous n'avons noté aucune différence entre les endroits étudiés relativement à l'abondance du périphyton. La physiologie du périphyton a été évaluée sur des échantillons étiquetés en double et incubés avec du bicarbonate marqué au ¹⁴C et des acides aminés marqués au ³H; on a ensuite analysé la répartition du carbone entre les sites lipidiques. L'apport total en éléments radiomarqués était équivalent pour tous les sites. En utilisant le rapport entre les lipides de la membrane et les lipides intracellulaires, on a obtenu un niveau maximal de stress physiologique à l'endroit le plus rapproché du point de rejet. Les valeurs élevées de ce rapport indiquaient que les composants phototrophes et hétérotrophes subissaient un stress, et que ce stress diminuait en fonction de la distance en aval. La capacité de déterminer l'abondance, la structure des communautés, l'activité et la condition physiologique des algues et des bactéries à partir d'une seule extraction représente une méthode efficace pour l'évaluation du périphyton, qui peut être utile comme outil de surveillance biologique et d'évaluation de la toxicité des écosystèmes aquatiques.

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Periphyton is the complex microbial biofilm which develops on submerged surfaces. Because of their importance in lotic ecosystems, periphyton analyses have been an important aspect of stream ecology research. In addition, their ubiquitous nature makes periphyton communities excellent integrating "monitors" for water quality in receiving rivers and streams (e.g. Weitzel 1979; APHA 1989; Boston et al. 1991).

Using periphyton for biological monitoring presents the same challenges as any in situ analysis of complex microbial communities (White 1985). The methods and measurements ("end-points") chosen to assess these communities determine the quality of results available for interpretation. Complex periphyton communities are often assessed with an emphasis on algal constituents (Aloi 1990), either from a viewpoint of biomass/community structure (e.g. Chessman 1985) or total photosynthesis (e.g. Blanck 1985). Periphytic bacterial populations are analyzed much less often (Dean-Ross 1990; Hudson et al. 1990), and only a few studies have monitored both algae and bacteria simultaneously (Stock and Ward 1989). Given the diversity of periphyton communities available in situ for water quality assessment studies, methods which provide an analysis of an entire assemblage might markedly improve resolution and sensitivity to anthropogenic chemicals.

The extraction of cellular lipids provides a quantitative and nonselective method of analysis for all viable microorganisms within complex communities (Vestal and White 1989). The fatty acid profiles of the plasma membrane phospholipids provide a multivariate index from all microbial populations (algal, bacterial, protozoan, fungal, etc.) within the community (Guckert and White 1986). Analysis of radiotracer incorporation into lipids can provide estimates of microbial activity, in terms of lipid synthesis, and physiological status, as carbon allocation patterns between storage lipids and membrane lipids (Findlay et al. 1990).

Total fatty acid profiles have been reported previously for laboratory stream periphyton; these profiles have distinct relationships to community structure (McIntire et al. 1969) and nutritional quality (Steinman et al. 1987, 1988). Although total fatty acid profiles are useful, even more information is available through fractionation of these into lipid classes. Algal lipids can be divided into three major classes: neutral lipids, which contain the storage lipid triglyceride; glycolipids, which generally have highly polyunsaturated fatty acids as the major membrane lipid of the chloroplast membranes; and polar lipids, which are dominated by the phospholipids from the plasma membrane (Guckert and Cooksey 1990). Fatty acid profile analyses in microbial ecology have focused on the polar lipid fraction due to its high extracellular turnover rate and constant relative amounts in microbial plasma membranes (Vestal and White 1989).

In this study, lipid analyses along with an algal/heterotrophic dual-label radiotracer were used as biochemical monitors to study periphyton physiological stress and community structure in an industrial receiving stream. This stream has been the focus of biological monitoring including periphyton, invertebrates, and biomarker analysis in fish.

Description of Study Site

East Fork Poplar Creek is a third-order stream located near the northern boundary of the Department of Energy's Oak Ridge National Laboratory (ORNL) Reservation in eastern Tennessee, USA. The headwaters of this creek lie within the Oak Ridge Y-12 nuclear weapons component manufacturing facility

where the creek receives diverse industrial effluents (see Hill 1992 for a map of the watershed). The algal, invertebrate, and fish communities in this creek have been studied extensively through a Biological Monitoring and Abatement Program to assess the possible effects of unique contaminants from the Y-12 plant (e.g. Loar et al. 1989; Stewart et al. 1990, 1992; Boston et al. 1991; Hinzman 1991).

East Fork Poplar Creek receives effluent from more than 200 individual discharges before leaving the Y-12 plant. The creek then flows into a 2.2-ha catchment basin designed for effluent pH equilibration, sediment retention, and spill control (Kingrea 1986). From the outfall of this basin, designated Lake Reality, the creek flows a distance of 23.7 km to its confluence with Poplar Creek, a tributary of the Clinch River. Study sites are designated by their kilometre distance above this confluence. Effluent discharges of $388 \text{ L} \cdot \text{s}^{-1}$ from the Y-12 plant constitute 25% of the mean annual flow of the creek at the 5.3-km gauging station. The stream also receives urban runoff and some agricultural runoff between sites 22.7 and 7.7 (Jimenez et al. 1990).

The water and sediments of East Fork Poplar Creek contain metals, organic chemicals, and radionuclides discharged during the operation of the Y-12 plant. However, monthly testing of water from the Lake Reality outfall (site 23.4) has provided little evidence for acute or chronic toxicity to *Ceriodaphnia* or fathead minnow (*Pimephales promelas*) larvae (Loar et al. 1989; Kszos et al. 1992). Whole-fish bioaccumulation results indicated high concentrations of contaminants such as mercury and PCBs that decreased with distance downstream (Adams et al. 1990; Stewart and Loar 1993). Community analyses and fish biomarker assessment also indicated poor biotic conditions at upstream sites (Loar et al. 1989; Jimenez et al. 1990).

Three sites along East Fork Poplar Creek were selected because they were similar with respect to physical parameters (ambient light intensities, stream flow, nutrient concentrations) but different with respect to biological Y-12 effluent effects (Loar et al. 1989). Site 24.4 is located upstream of Lake Reality. Chlorine concentrations at this site often exceed $150 \mu\text{g} \cdot \text{L}^{-1}$ (A. J. Stewart, ORNL, pers. comm.). Algal periphyton is physiologically stressed and exhibits low species diversity (Boston et al. 1991). Few fish or invertebrate species have been found at this site (Loar et al. 1989).

Site 23.2, located about 500 m downstream from Lake Reality, is characterized by greater periphytic algal taxonomic diversity than site 24.4; however, pollution-sensitive fish and invertebrate species are absent (Loar et al. 1989). Fish that are present at this site are physiologically stressed relative to unpolluted reference sites, based on biomarkers such as induction of the hepatic mixed-function oxidase system components (Adams et al. 1990; Jimenez et al. 1990) and carbohydrate/protein metabolism and membrane/storage lipid metabolism (Adams et al. 1990).

At site 13.8 the biological communities and physicochemical conditions are typical of nonpolluted streams in eastern Tennessee. The invertebrate communities contain fewer oligochaetes and have about 10 times more insect biomass (areal basis) than the upstream sites (Loar et al. 1989). Additionally, the fish are less stressed physiologically than they are in the upstream sites (Adams et al. 1990; Jimenez et al. 1990).

Materials and Methods

Periphyton Sampling

At each sampling site, 20 relatively flat small rocks (average $\pm$ SD upper surface area = $45 \pm 12 \text{ cm}^2$) with their attached

periphytic communities were collected from shallow riffles (<30 cm deep) on each of three dates (March 22, April 4, and May 9, 1990). The rocks were immediately placed in opaque containers with water from the collection site for transport to the laboratory.

Eight rocks from each site were randomly chosen and placed into a 2-L recirculating chamber with 1 L of stream water from that site within 30 min of collection. The recirculation chambers have been described elsewhere (Mulholland et al. 1986; Boston and Hill 1991). The remaining 12 rocks were stored in the dark at approximately in situ temperatures (15–20°C) for no more than 2 h for the following assays: bacterial cell density, organic carbon content, and algal taxonomic composition.

Radiolabel Incubation

The rocks within the recirculating chambers were incubated under constant illumination (approximately 400 $\mu\text{mol quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiation, $\approx 16\%$ full sun) provided by a 1000-W metal halide lamp. During the incubation, the water temperature was maintained within 4°C of ambient stream temperature and the water in the incubation chambers was circulated constantly by submersible pumps to simulate natural conditions (Boston and Hill 1991).

We used a dual-label incubation method to simultaneously measure photoautotrophic (^{14}C -labeled dissolved inorganic carbon (DIC)) and heterotrophic (^3H -labeled dissolved organic carbon (DOC)) radiolabel uptake/incorporation. Initially, 10 μCi of $\text{NaH}^{14}\text{CO}_3$ (1 $\mu\text{Ci} = 37 \text{ kBq}$) (specific activity = 1.2 $\text{mCi}\cdot\text{mmol}^{-1}$, New England Nuclear NEC-086S) was added to each chamber. After 45 min, 20 μCi of a mixture of amino acids (approximately 54 $\text{mCi}\cdot\text{mmol}^{-1}$, New England Nuclear NEC-250) was added. The rocks were removed after an additional 45 min and rinsed twice in distilled water just prior to extraction.

DMSO Extraction

Four of the rocks from the recirculating chambers were each placed in a container with 30 mL of dimethylsulfoxide (DMSO) in the dark for 24 h. The material extracted by this technique was used to simultaneously measure chlorophyll *a*, ATP, [^{14}C] DIC uptake/incorporation and [^3H] DOC uptake/incorporation for each replicate rock (Palumbo et al. 1987).

Chlorophyll *a* was used as an estimate of algal biomass. Five milliliters of DMSO extract was diluted 1:1 with acetone and chlorophyll *a* concentration determined spectrophotometrically with corrections made for phaeopigments (Boston et al. 1991).

ATP was measured to provide an estimate of community biomass. ATP was assayed in an aliquot of DMSO extract using Turner reagents and a Turner ATP photometer. Phosphate was added to DMSO (20 mM final concentration) prior to extraction to prevent loss of ATP due to phosphatase activity (Palumbo et al. 1987).

^{14}C and ^3H contents in the DMSO extract were determined by liquid scintillation counting (LSC). A 500- μL aliquot of extract was added to 10 mL of scintillation cocktail (AquaSol, New England Nuclear) and assayed in a Packard Tri-Carb scintillation spectrometer using a dual-label protocol. The protocol used an external standard ratio quench correction, and counting times continued until a constant variance was achieved ($\sigma = 1.5\%$).

Lipid Extraction

The remaining four rocks from each recirculating chamber

were extracted for lipids using a chloroform/methanol/phosphate buffer procedure as described in Guckert et al. (1985). Following phase separation, a 1-mL aliquot was removed from the aqueous phase and added to 10 mL of AquaSol for LSC. The organic (lipid) phase was drained into a round-bottom flask for removal of solvents under vacuum (<37°C). Ten percent of the total lipid was then removed to a LSC vial. The samples were photobleached (1000-W metal halide lamps used in incubation) overnight prior to AquaSol (10 mL) addition to reduce color quench for LSC. The dual-label LSC protocol was used for all lipid LSC. The remaining lipid was stored under nitrogen at -20°C for later analysis.

The total lipids were separated into three general lipid classes by silicic acid column chromatography (0.5 g of Unisil, 100–200 mesh) using a series of mobile phases of increasing polarity: neutral lipids, 10 mL of chloroform; glycolipids, 10 mL of acetone; and polar lipids, 10 mL of methanol (Guckert et al. 1985). Following solvent removal under a nitrogen purge, a 10% aliquot from each lipid class was removed for dual-label LSC as described above. Fatty acid methyl esters were prepared from the esterified lipids in the polar lipid fraction by mild alkaline methanolic transesterification (Guckert et al. 1985). Methods for the separation, quantification, and identification of the fatty acids in each lipid class by capillary gas chromatography (GC) and mass spectrometry are described in Guckert and Cooksey (1990) and Guckert et al. (1985). Equivalent chain lengths were used to assist in the identification of fatty acid methyl esters by GC (Kates 1986).

Fatty acid nomenclature used in this study was of the form “A:B ω C” where “A” designates the total number of carbon atoms, “B” the number of double bonds, with the position closest to the aliphatic (ω) end of the molecule indicated as “C” with the geometry suffix “c” for *cis* and “t” for *trans*. The prefixes “i” and “a” refer to iso and anteiso methyl-branching, respectively.

Bacterial Density and Organic Carbon Content

Four of the rocks collected and stored in the dark for each study site were used for these assays. Periphyton was brushed from the rock surfaces with a toothbrush into a known volume of water. This periphyton suspension was diluted 1:10 with distilled water for bacterial cell enumeration with acridine orange stain (ASTM 1987). Equal parts of diluted suspension and acridine orange stain (0.01%, w/v) were mixed and filtered through a black polycarbonate membrane filter (Nuclepore 0.2- μm pore size). Stained bacteria were counted in 20 grids at 1000 $\times$ power using a Nikon Labophot microscope equipped with a mercury vapor lamp (HBO 50 W). Organic carbon content was also determined as a measure of community biomass for these rocks. A 50-mL aliquot of the above periphyton suspension was filtered through an ashed, preweighed Whatman G6 glass fiber filter. The filter was dried for 24 h at 65°C and weighed. The filter was then combusted at 500°C for 1 h, after which ash weight was determined (APHA 1989).

Algal Taxonomic Composition

Periphyton was brushed from the remaining rocks stored after collection and examined microscopically to provide a qualitative assessment of algal taxonomic composition.

Rock Surface Areas

The area of the rock that was covered with periphyton was determined by covering the upper surface of the rock with alu-

minum foil, determining the weight of the foil, and converting to surface area based on a known weight per unit area of foil (Boston et al. 1991). This procedure was repeated twice for each rock. All parameters, except the fatty acid profiles, were normalized to periphyton (top) area; fatty acid profiles were normalized to the entire rock surface area.

Statistical Analysis

Data are presented as arithmetic means with the standard deviations. Differences among sites were evaluated with an ANOVA (mainframe SPSSx Version 3.0). Ratio data (e.g. membrane to storage lipid ratios and fatty acid mole percents) were arcsin-square root transformed before testing (Winer 1971). Multivariate analysis of fatty acid profiles was done with the pattern recognition software package Ein*Sight Version 2.5 (Infomatrix, Inc.). Dendrograms were constructed with the Ein*Sight cluster analysis using a complete linkage, farthest neighbor method as well as all other clustering algorithms available with Ein*Sight or SPSSx. For principal component analysis, the raw fatty acid mole percent data were first autoscaled to a mean of 0 and constant variance. Loadings were generated for both the samples and the individual fatty acids in the profile.

Results

Periphyton Abundance and Activity

Periphyton biomass estimates (ATP, chlorophyll *a*, bacterial cell density) were conducted over the first 7 mo of 1990 for sites 24.2, 23.3, and 13.8. Algal periphyton coverage averaged $49.5 \pm 10.3\%$ of the total rock surface area at all sites. No consistent trends in biomass estimates between sites were evident. The chlorophyll content was $10\text{--}30 \mu\text{g Chl } a \cdot \text{cm}^{-2}$ and bacterial cell density was consistently about 3×10^{10} cells $\cdot \text{cm}^{-2}$ when sampled in March, April, and May (Fig. 1). Photosynthetic carbon (^{14}C in DMSO) fixation varied over the times sampled ($18\text{--}96 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$). There was a trend for rates to be lower at the impacted upstream site 24.4, but most of the differences were not significant (Fig. 1). The rate of algal activity (both photosynthesis and total lipid synthesis) was higher ($\alpha = 0.05$) at the nonpolluted site 13.8 on April 4 as compared with the impacted site 24.4 (Fig. 1). The date of this increase corresponded to an observed *Gomphenema* bloom at site 13.8 (W. R. Hill, ORNL, unpubl. data).

Heterotrophic uptake (^3H content of either DMSO or total lipid extracts) was consistent over all sites and times. The heterotrophic community assimilated approximately $0.35 \text{ pmol amino acids} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ (Fig. 1).

Evaluating Physiological Stress

The radiolabel content of the total lipid extracts (Fig. 1) is useful for comparisons with the DMSO extracts. When the dpm recovered in DMSO was compared with the dpm recovered in lipids, there was $6.3 (\pm 5.3)$ times more ^3H in DMSO than in lipids and $2.0 (\pm 0.8)$ as much ^{14}C . This value decreased when the dpm recovered in the aqueous phase of the lipid extraction was included (dpm DMSO/dpm lipid extraction ratios: 2.4 ± 2.6 for ^3H , 1.5 ± 0.6 for ^{14}C).

The lipid assays also provided additional information by evaluating the radiotracer incorporation into lipid classes. Virtually 100% of both ^3H ($106 \pm 19\%$) and ^{14}C ($102 \pm 9\%$) applied to the silicic acid columns as total lipid extracts was recovered

in the neutral, glyco-, and polar lipid classes. Since total lipid synthesis results agreed with DMSO results and there was no loss of material during lipid class separation, the lipid class patterns provide an analysis of carbon allocation within the periphyton.

Examination of the phototrophic lipid class patterns (defined as the ^{14}C content of lipid classes) by study site and sampling revealed that the plasma membrane polar lipid ^{14}C content was about the same for all samples (Fig. 2). Periphyton from site 24.4 had smaller amounts of ^{14}C in the storage neutral lipids relative to the other sites. Sites 23.2 and 13.8 had larger, but variable, quantities of ^{14}C in the neutral lipid fraction. At all sites, the amount of ^{14}C incorporated into chloroplast membrane glycolipid exceeded the amount of ^{14}C in the polar lipid fraction (Fig. 2).

The heterotrophic community consistently incorporated the majority of the ^3H from amino acids (DOC) into plasma membrane polar lipids (Fig. 2). The ^3H content of polar lipids was similar for all sites and all times. Bacteria generally do not contain neutral lipids, although we always detected some ^3H in this lipid class at site 13.8. Bacterial storage lipids were found in the glycolipid fraction, principally in the form of poly- β -hydroxybutyrate. The ^3H content of this lipid class was highly variable, but tended to be higher for sites 23.2 and 13.8 than for site 24.4.

The heterotrophic membrane to storage lipid ratios were defined as the ^3H content of the polar lipid fraction to the glycolipid fraction. The phototrophic ratios were defined as the ^{14}C content of the polar and glycolipid fractions to the neutral lipid fraction. We found a consistent and statistically significant ($\alpha = 0.05$) longitudinal trend for both the heterotrophic and phototrophic membrane to storage lipid ratios, with the highest values occurring in periphyton from site 24.4 in each sampling period (Fig. 3).

Periphyton Community Structure

Periphyton fatty acid profiles were analyzed in a three-step process for community structure assessment: (1) data set exploration using multivariate cluster analysis to suggest structure in the data set; (2) fatty acid profiles (average $\pm$ SD) reported for grouped data; and (3) multivariate principal components analysis for the final analysis.

The periphyton fatty acid profiles were grouped by cluster analysis based primarily on location in the stream. Since the periphyton fatty acid profile (= community structure) appeared to be most consistent by study site over the sampling period, fatty acid profiles were grouped by site to investigate trends (Table 1). Total fatty acid content of the periphyton was not significantly different for any site, which was consistent with other biomass/abundance estimates. The fatty acid profiles of site 24.4 were dominated by 16- and 18-carbon fatty acids such as 18:1 ω 9c (31%), 16:0 (20%), 16:1 ω 7c (10%), 18:2 ω 6 (9%), and 18:1 ω 7c (6%) (Table 1). Site 23.2 also contained high levels of 16:1 ω 7c (20%) and 16:0 (17%), along with equivalent amounts of the long-chain polyunsaturated fatty acids 20:4 ω 6 (10%) and 20:5 ω 3 (10%). In addition, the profiles from this site contain fatty acids common to bacterial membranes such as i18:0 (6%), 16:1 ω 5c (6%), and 18:1 ω 7c (5%) (Table 1). A fatty acid common in diatom membranes, 20:5 ω 3, dominated the profiles of site 13.8 (23%). Other major fatty acid included 16:0 (18%), 16:1 ω 7c (12%), i18:0 (7%), and 18:2 ω 6 (5%).

Principal components analysis was used to view the discrimination of the East Fork Poplar Creek periphyton communities

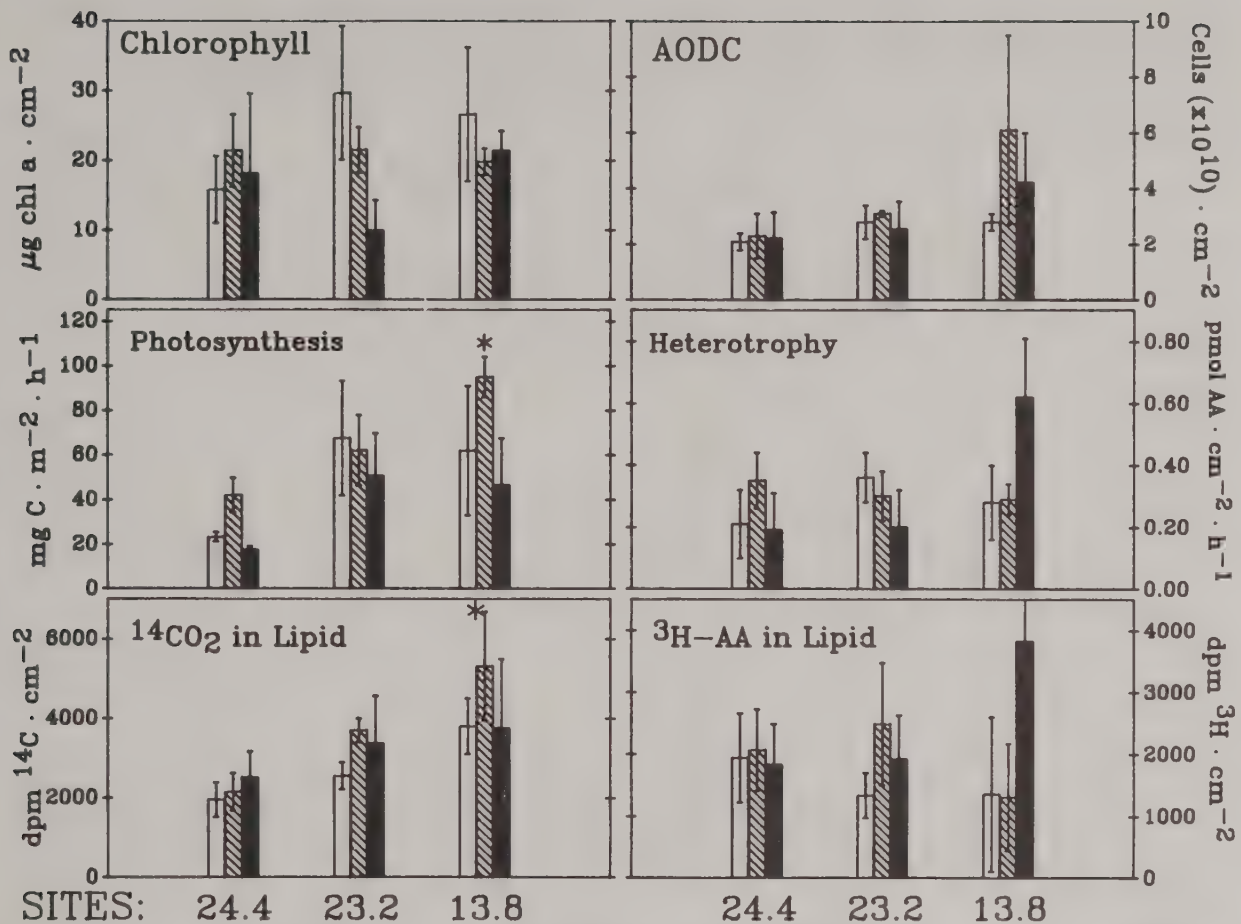


Fig. 1. Average ($\pm$ SD) East Fork Poplar Creek periphyton biomass and activity estimates for March 22 (open bars), April 4 (hatched bars), and May 5 (solid bars), 1990, samplings. Algal periphyton values (chlorophyll, photosynthesis, $^{14}\text{CO}_2$ in lipid) are shown in the left-hand panels. Heterotrophic values (cell counts by AODC, heterotrophy, ^3H in lipid) are on the right. An asterisk indicates a value significantly different ($\alpha = 0.05$) from the corresponding value at site 24.4.

by fatty acid profiles and to evaluate the influence of fatty acid patterns in the separation of study sites. The separation of the replicates by the first two principal components (PC) (Fig. 4) accounted for 71% of the variability within the data set (PC1 = 51%, PC2 = 20%). The third PC accounted for 8% of the variability, and a scree plot analysis indicated that no further information would be gained by evaluating the fourth principal component (PC4 = 6%). The study sites separated clearly based on PC1 and PC2 (Fig. 4). This separation was essentially identical to that achieved by cluster analysis.

The loadings of the fatty acids for PC1-separated site 24.4 in a positive loading (Fig. 4) were highly positive with fatty acids common to green algae (18:1 ω 9c, 16:4 ω 3, 18:2 ω 6, and 16:1 ω 13t) (Table 2). PC2, which separated site 13.8 (positive loadings) and site 23.2 (negative loadings) (Fig. 4), had positive fatty acid loadings commonly found in diatoms and other microeukaryotes (22:4 ω 6, 14:0, 20:5 ω 3, 18:3 ω 6) and negative loadings with mostly bacterial monounsaturated fatty acids (16:1 ω 5c, 16:1 ω 7c, 18:1 ω 5c) as well as 20:4 ω 6 (Table 2). PC3, which separated the April 4 samples of sites 23.2 and 13.8 from the other sampling times (negative loadings, data not shown), was dominated by 22:6 ω 3 in the negative direction, although

other diatom fatty acids such as 20:5 ω 3 were also contributing to the separation (Table 2).

Discussion

Periphyton communities are useful for the evaluation of aquatic toxicity (Boston et al. 1991). The periphyton communities of East Fork Poplar Creek were shown to be physiologically stressed at the upstream site 24.4 when compared with other sites. The stress, as measured with a membrane to storage lipid synthetic ratio for both the phototrophic and heterotrophic constituents, declined with distance downstream from the source of the industrial effluents and was consistent over three sampling periods. These results agreed with previous periphyton stress analyses conducted at these sites (Boston et al. 1991). This work suggests that the use of lipid-based assays along with a simultaneous phototroph/heterotroph activity analysis can provide further insight into this ecologically important community. This detailed information will improve the use of periphyton as a biomonitoring and aquatic toxicity assessment tool.

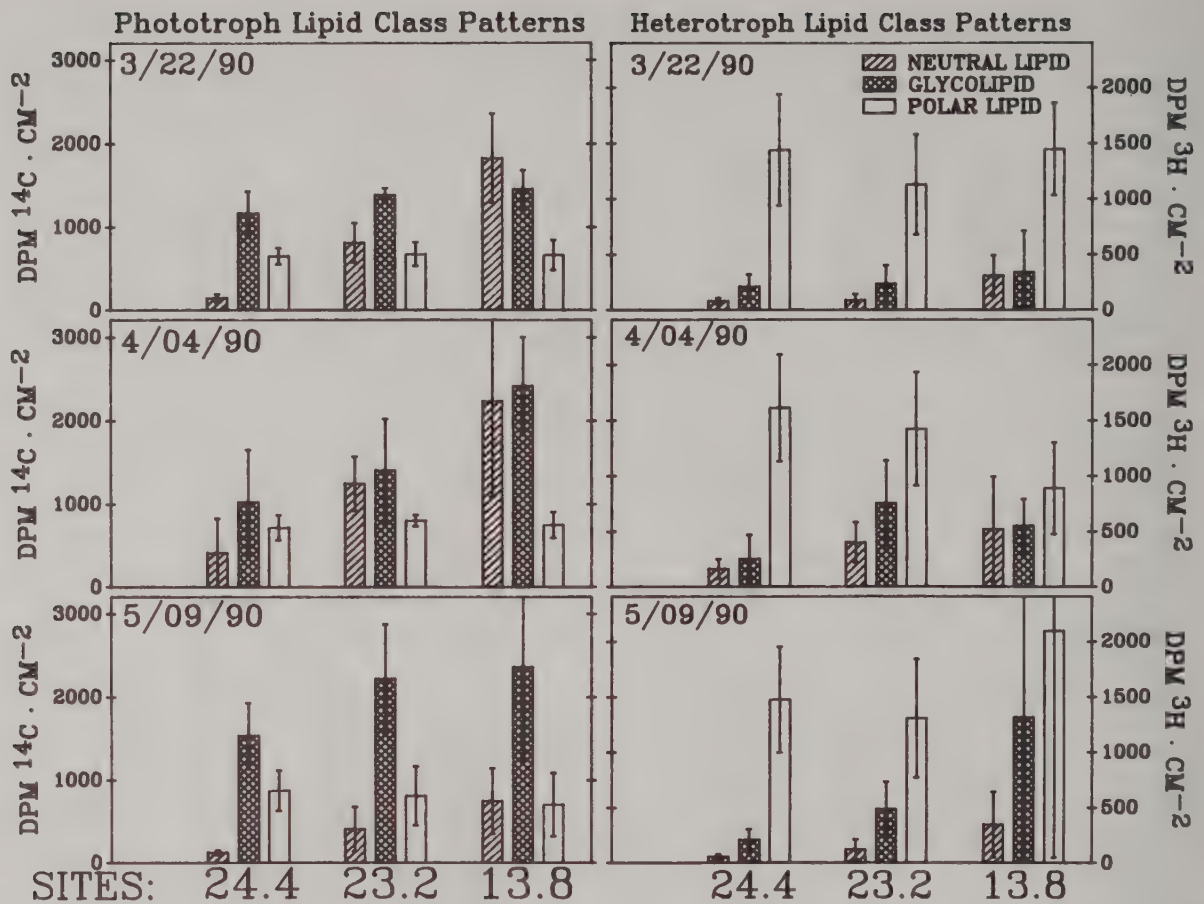


FIG. 2. Average ($\pm$ SD) East Fork Poplar Creek periphyton lipid class radiolabel incorporation patterns including neutral lipids (hatched bars), glycolipids (cross-hatched bars), and polar lipids (open bars). Phototrophic (^{14}C incorporation) patterns for the three sampling dates are shown in the left-hand panels. Heterotrophic (^3H incorporation) patterns are on the right. Sample dates are indicated for each set of graphs.

Evaluating Physiological Stress

Microbial activities are often measured by the quantity of radiotracer carbon assimilated over time. Physiological status must then be inferred by a comparison of activities or the calculation of biomass-normalized activities (e.g. chlorophyll-adjusted photosynthetic rates (Boston et al. 1991)). Analysis of radiotracer carbon assimilated into total lipid is an additional measure of activity, and its partitioning into lipid classes provides an improved method to estimate physiological status.

Other macromolecules could be evaluated for carbon allocation such as proteins or carbohydrates (Amblard et al. 1990). An evaluation of membrane versus storage lipid synthesis rates, however, has the advantage of using a common lipid precursor pool. This simplifies physiological interpretations because differences of radiotracer incorporation into separate macromolecules can be both a function of synthetic rates and differences in precursor pool sizes. Also, the membrane and storage lipid information can be obtained simultaneously from one extraction, which simplifies the procedures.

Increased membrane to storage lipid synthesis ratios have been previously reported for bacterial responses to physical perturbations in stratified estuarine sediments (Findlay et al. 1990). This same response can be detected for both phototrophic and

heterotrophic benthic microbial communities in the work of Dobbs et al. (1989) if their data are replotted as membrane to storage lipid ratios versus level of disturbance due to radiolabel introduction into sediment cores (injection < pore-water replacement < slurry). Our results with periphyton are the first to describe the increased membrane to storage lipid ratios for microbial communities exposed to industrial effluent.

Elevated membrane to storage lipid ratios due to toxicant exposure have been reported in macrofauna. Fraser (1989) reported increased membrane sterol to triglyceride ratios in both juvenile lobsters and blue crabs with increasing concentrations of crude oil. The most significant example for this work, however, are the changes in lipid content which occur in East Fork Poplar Creek fish with distance downstream. Adams et al. (1990) reported decreases in phospholipid and increases in triglyceride (i.e. membrane to storage lipid ratios decline) for fish caught near site 13.8 when compared with fish caught at several sites further upstream. These results suggest that the microbial membrane to storage lipid ratios might be predictive of similar macrofaunal responses to water quality.

There are ecological implications of consistently high membrane to storage lipid synthetic ratios in periphyton communities. Periphyton biomass (abundance) was similar for the

Membrane to Storage Lipid Ratios

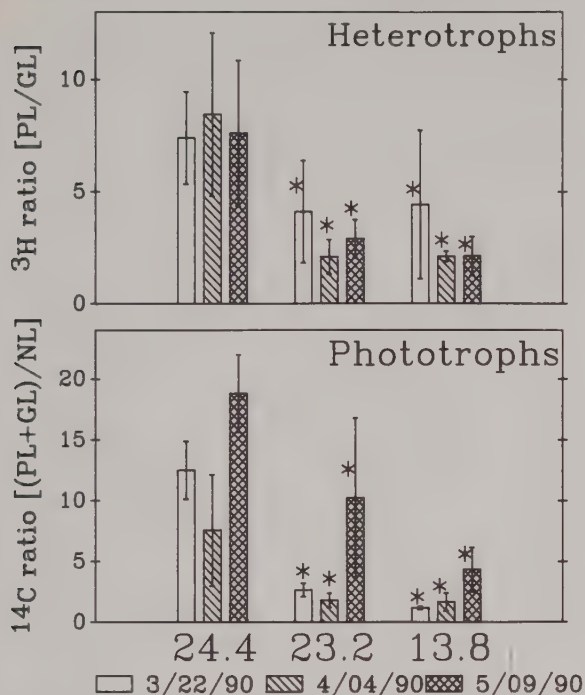


Fig. 3. Average ($\pm$ SD) for heterotrophic (^3H ratio on top) and phototrophic (^{14}C ratio on bottom) membrane to storage lipid synthetic ratios (PL = polar lipids; GL = glycolipids; NL = neutral lipids). Values are given by East Fork Poplar Creek site and sampling date (March 22 = open bars, April 4 = hatched bars, May 5 = cross-hatched bars). All values for sites 23.2 and 13.8 were significantly lower ($\alpha = 0.05$, marked with an asterisk) than site 24.4 values on each sampling day.

three study sites (Fig. 1; Table 1), and total carbon uptake/incorporation had only a slight trend of increase with distance downstream. The communities of the impacted upstream site 24.4 were, therefore, consistently using a higher proportion of their assimilated carbon for membrane synthesis without subsequent increases in biomass. A community under chronic stress would be expected to increase its carbon allocation to biomass components at the expense of storage materials (Odum 1985). As a consequence, the periphyton communities of site 24.4 may be more vulnerable to other stresses and perturbations due to lower energy reserves. In addition, since periphyton serves as an important food resource in streams, communities not accumulating as much carbon in lipid storage pools are more likely to be of lower food quality to grazers. In fact, Hill (1992) reported that periphyton from East Fork Poplar Creek site 13.8 (site EF2 in his study) is a better food resource than periphyton from site 23.2 for the snail *Elimia clavaeformis* and the caddis fly larva *Neophylax etnieri*. In a laboratory feeding experiment, snails fed periphyton from site 13.8 tended to have higher growth rates and neutral lipid content than those fed periphyton from site 23.2. Caddis fly larvae had similar trends along with a significantly faster developmental cycle when fed periphyton from site 13.8 (Hill 1992).

Heterotrophic Activities within Periphyton

Another important aspect of this work was the simultaneous evaluation of phototrophic (^{14}C -labeled bicarbonate) and het-

TABLE 1. Phospholipid, ester-linked fatty acid profiles of East Fork Poplar Creek periphyton by study site ($n = 12$). Results are expressed as average ($\pm$ SD) percentages of total fatty acid recovered.

Fatty acid ^a	ECL ^b	Site 24.4	Site 23.2	Site 13.8
14:0	14.00	0.8 $\pm$ 0.3	2.0 $\pm$ 0.7	4.2 $\pm$ 1.2
16:4 ω 3	15.47	1.3 $\pm$ 0.2	0.4 $\pm$ 0.1	0.3 $\pm$ 0.2
16:0	15.63	1.1 $\pm$ 0.1	0.5 $\pm$ 0.3	0.3 $\pm$ 0.2
16:1 ω 7c	15.75	9.8 $\pm$ 1.7	19.7 $\pm$ 2.2	11.9 $\pm$ 1.9
16:1 ω 5c	15.84	1.6 $\pm$ 0.9	6.5 $\pm$ 1.9	0.9 $\pm$ 0.2
16:1 ω 13t	15.92	4.1 $\pm$ 0.5	2.0 $\pm$ 0.3	1.8 $\pm$ 0.4
16:0	16.00	20.5 $\pm$ 2.0	16.9 $\pm$ 1.9	18.0 $\pm$ 3.5
Unknown	17.43	0.3 $\pm$ 0.1	0.9 $\pm$ 0.1	1.2 $\pm$ 0.3
18:3 ω 6	17.46	2.8 $\pm$ 0.4	1.5 $\pm$ 0.2	2.4 $\pm$ 0.5
18:2 ω 6	17.62	8.6 $\pm$ 0.7	3.6 $\pm$ 0.8	5.0 $\pm$ 1.3
18:0	17.66	0.0 $\pm$ 0.0	6.1 $\pm$ 2.0	6.8 $\pm$ 2.9
18:1 ω 9c	17.71	30.9 $\pm$ 2.8	2.5 $\pm$ 0.4	4.5 $\pm$ 2.2
18:1 ω 7c	17.76	5.9 $\pm$ 1.1	5.4 $\pm$ 1.3	4.7 $\pm$ 1.0
18:1 ω 5c	17.86	1.0 $\pm$ 0.3	1.0 $\pm$ 0.2	0.7 $\pm$ 0.2
18:0	18.00	2.1 $\pm$ 0.6	1.3 $\pm$ 0.4	2.0 $\pm$ 0.8
20:4 ω 6	19.21	1.7 $\pm$ 0.6	9.8 $\pm$ 3.2	4.0 $\pm$ 1.0
20:5 ω 3	19.28	3.0 $\pm$ 0.8	9.8 $\pm$ 2.2	22.8 $\pm$ 8.5
22:6 ω 3	21.10	0.0 $\pm$ 0.0	0.8 $\pm$ 0.3	1.0 $\pm$ 0.9
22:4 ω 6	21.24	0.0 $\pm$ 0.0	0.5 $\pm$ 0.2	1.5 $\pm$ 0.7
Σ nmol $\cdot$ cm $^{-2}$		28.0 $\pm$ 8.0	32.7 $\pm$ 9.6	29.5 $\pm$ 9.3

^aPhospholipid, ester-linked fatty acids.

^bEquivalent chain length.

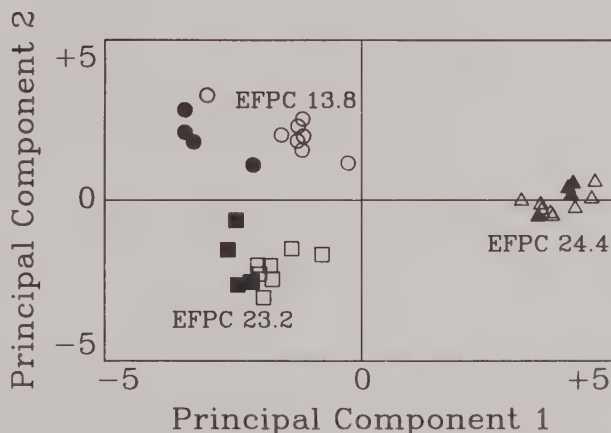


Fig. 4. Sample loadings for the first two principal components (PC) of East Fork Poplar Creek (EFPC) periphyton phospholipid fatty acid profiles. Site 24.4 (triangles) is separated from the other downstream sites by PC1. Sites 23.2 (squares) and 13.8 (circles) are more closely related, but are separated by PC2. The April 4 samples are represented by solid symbols.

erotrophic (^3H -labeled amino acids) activity. Amino acid mixtures have been shown to be readily utilized by bacterial periphyton (Dean-Ross 1990). Our use of [^{14}C] DIC and [^3H] DOC provides a more complete view of periphyton activities and physiological status.

Few prokaryotic heterotrophs synthesize significant amounts of neutral lipid. The source of the ^3H recovered in the neutral lipid fraction from site 13.8 is unknown. Additional time course analyses have indicated that this pattern is established early in the incubation time course (within 5 min), suggesting that potential eukaryotic direct incorporation rather than incorporation into a bacteriovore may be occurring (S. C. Nold, unpubl. data).

TABLE 2. Principal component (PC) loadings for fatty acid. Highest loading is highlighted (asterisk) for each PC. Loading value corresponds to placement onto the PC axes.

Fatty acid	PC1	PC2	PC3
18:1 ω 9c	+0.314*	+0.045	-0.133
16:4 ω 3	+0.305*	+0.0005	-0.115
18:2 ω 6	+0.301*	+0.140	+0.072
16:1 ω 13t	+0.299*	-0.038	-0.186
i16:0	+0.284*	-0.121	-0.047
Unknown	-0.270*	+0.096	+0.131
16:1 ω 5c	-0.113	-0.443*	+0.059
16:1 ω 7c	-0.204	-0.376*	-0.042
22:4 ω 6	-0.195	+0.313*	+0.144
14:0	-0.225	+0.302*	+0.084
20:4 ω 6	-0.215	-0.292*	-0.088
18:1 ω 5c	+0.054	-0.286*	+0.109
20:5 ω 3	-0.238	+0.284*	-0.197
18:3 ω 6	+0.198	+0.274*	-0.070
i18:0	-0.228	+0.031	+0.497*
18:1 ω 7c	+0.144	-0.126	+0.487*
16:0	+0.202	+0.020	+0.400*
22:6 ω 3	-0.236	+0.127	-0.316*
18:0	+0.130	+0.263	+0.264*

Periphyton Community Structure based on Lipids

Another benefit of the lipid-based method is that periphyton community structure, based on fatty acid profiles, can be obtained with little additional effort. Algal communities can be evaluated taxonomically, but much of the microbial community (e.g. bacteria) cannot be identified or quantified accurately. Thus, fatty acid profiles can serve at least three purposes in periphyton evaluations, as follow.

(1) Fatty acid profiles are nontaxonomic descriptions, but comparable in data structure with algal taxonomic lists (McIntire et al. 1969). The fatty acid profiles can be used to confirm trends noted in the taxonomic evaluations, such as the green algal dominance at site 24.4 and the diatom dominance at site 13.8.

(2) The fatty acid profiles provide a quantitative index of the entire periphytic community, which is not available by any other method. This includes the heterotrophic bacteria which, especially for site 23.2, had not been previously recognized as being significantly different from the other sites as indicated by the multivariate analysis (Fig. 4; Table 2). For the East Fork Poplar Creek periphyton, the fatty acid analysis indicated stable separation of community structure by study site. The most impacted site (24.4) was easily separated from other downstream sites. Interestingly, the *Gomphenema* bloom on April 4 only resulted in a temporary change in the site 13.8 fatty acid profiles which was absent at the next sample period (Fig. 4). The stability of the fatty acid profiles suggests that patterns of these profiles may be used to classify the degree of chronic impact for any given site.

(3) The fatty acid profiles may be useful for discussing food quality issues (although total lipid profiles would be more appropriate). The profiles shown in Table 1 and loadings shown in Table 2 suggest that for the same amount of biomass, the periphyton of site 13.8 is clearly superior as a source of long-chain (>18 carbons) ω -3 polyunsaturated fatty acids. This is not a definitive measure of food quality but does suggest an important hypothesis to test regarding the increased grazer biomass at this site and may help to explain the results of Hill (1992) which showed that a diet of periphyton from East Fork

Poplar Creek site 13.8 improved growth, development, and neutral lipid content in the grazers.

In conclusion, the lipid analyses outlined here for radio-tracer-incubated stream periphyton would be an excellent complement to more traditional endpoints evaluated. The lipid data will help to confirm and refine results for total DMSO-soluble material (biomass, activity) as well as algal taxonomy (community structure). Physiological status of the periphyton can be obtained through the carbon allocation patterns of membrane and storage lipids. This level of biological evaluation is essential if periphyton is to further develop into a biomonitoring and toxicity assessment tool for aquatic ecosystems.

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Survival of Northern Atlantic Cod (*Gadus morhua*) Eggs and Larvae when Exposed to Ice and Low Temperature

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Valerio, P. F., S. V. Goddard, M. H. Kao, and G. L. Fletcher. 1992. Survival of northern Atlantic cod (*Gadus morhua*) eggs and larvae when exposed to ice and low temperature. *Can. J. Fish. Aquat. Sci.* 49: 2588–2595.

Freeze resistance of eggs and larvae of Atlantic cod (*Gadus morhua*) from the northern cod stock was investigated to determine whether ice contact could affect survival during the spring spawning season off Newfoundland. Egg and larval homogenates did not appear to contain antifreeze proteins (mean freezing points -0.78 and -0.88°C , respectively). However, cod eggs did not freeze at -1.8°C in icy aerated seawater, could be undercooled to -4.0°C in ice, and froze at temperatures between -4.1 and -17°C ; freeze resistance depended on the integrity of the chorion. Larvae withstood undercooling to -1.8°C , provided they were not brought into direct contact with ice crystals. If directly touched with ice, larvae froze at -1.36°C (feeding stage) or -1.34°C (yolk-sac), approximately 0.5°C lower than would be expected from the freezing temperatures of their body fluids. The nature of their external epithelium and delayed development of sensitive gill structures below 0°C may contribute to larval freeze resistance. Cod eggs and larvae are found in spring off Newfoundland and Labrador, when sea temperatures can be as low as -1.8°C and ice cover extensive. While cod eggs are remarkably freeze resistant, such environmental conditions may cause freezing mortalities in larval cod.

Nous avons étudié la résistance au froid des oeufs et des larves de morue franche (*Gadus morhua*) provenant du stock nordique de morues afin de déterminer si le contact avec la glace pourrait influencer sur la survie au cours de la saison printanière de reproduction au large de Terre-Neuve. Les homogénats d'oeufs et de larves ne semblaient pas contenir de protéines antigels (le point de congélation moyen étant à $-0,78$ et à $-0,88^{\circ}\text{C}$ respectivement). Toutefois, les oeufs de morue n'ont pas gelé à $-1,8^{\circ}\text{C}$ dans l'eau de mer aérée et glacée, et après avoir été refroidis à $-4,0^{\circ}\text{C}$ dans la glace, ils ont gelé entre $-4,1$ et -17°C ; la résistance au froid était tributaire de l'intégrité du chorion. Les larves ont toléré le refroidissement à $-1,8^{\circ}\text{C}$ à la condition de ne pas être en contact direct avec les cristaux de glace. Lorsque les larves touchaient directement à la glace, elles gelaient à $-1,36^{\circ}\text{C}$ (larves ayant perdu le sac vitellin et se nourrissant librement) ou à $-1,34^{\circ}\text{C}$ (se nourrissant à partir du sac vitellin), soit environ $0,5^{\circ}\text{C}$ de moins que l'on prévoyait d'après le point de congélation de leurs liquides organiques. Chez ces larves, la nature de l'épithélium externe et le développement retardé des structures branchiales sensibles en dessous de 0°C peuvent contribuer à la résistance au froid. Les oeufs et les larves de morues franches sont présentes au large de Terre-Neuve et du Labrador au printemps, quand la température de la mer peut descendre même jusqu'à $-1,8^{\circ}\text{C}$ et que le manteau glaciaire est très étendu. Bien que les oeufs de morue soient remarquablement résistants au froid, des conditions environnementales semblables peuvent causer la mort par le gel des larves de morue.

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Adult Atlantic cod (*Gadus morhua*) of the northern cod stock (NAFO Divisions 2G, 2J, 3K, L) overwinter in deep water on the outer slopes of the banks of Newfoundland and Labrador, where they form spawning aggregations in early spring (Templeman and May 1965; Templeman 1979; Lear and Green 1984). Evidence from surveys of cod eggs and larvae, and the reproductive status of adult cod gonads, indicates that spawning takes place first in cod inhabiting the most northerly regions (2G, 2J) and progressively later in the spring with decreasing latitude (Serebryakov 1965, 1967; Postolaky 1963, 1968; Templeman 1981; Lear and Green 1984; C. Bishop, DFO, St. John's, Nfld., pers. comm.).

Spawning occurs near the seabed at depths greater than 250 m, at temperatures around $2\text{--}4^{\circ}\text{C}$ (Lear and Green 1984). The eggs are buoyant and ascend to the surface where they encounter the cold waters of the Labrador current (Serebryakov 1965). Cod eggs and larvae are found above 60 m depth, with the majority occurring in the upper 30 m of the water column

(McKenzie 1940; Serebryakov 1965; Templeman 1981; Ellertsen et al. 1989; Sundby and Sunnanå 1990; J. Anderson, DFO, St. John's, Nfld., pers. comm.). In the Newfoundland and Labrador region, temperatures in the top 30 m can approach the freezing point of seawater (-1.8°C). These waters are often covered by ice in spring, occasionally remaining icy into June (Symonds 1986; Fissel et al. 1989). Because cod eggs and larvae are found off the coast of Labrador as early as February and April, respectively (Serebryakov 1965, 1967; Postolaky 1963, 1968), it is apparent that early cod development may occur at subzero temperatures in intimate association with ice, an environment that is lethal to most fish species (Fletcher et al. 1986; Davies et al. 1988).

A number of studies have established that postmetamorphic teleost fish freeze and die when they come into contact with ice at temperatures below the freezing point of their blood plasma (DeVries and Lin 1977; Fletcher et al. 1986, 1988). For most teleosts, the plasma freezing point ranges from -0.6

to -0.8°C depending on body fluid electrolyte concentration (Holmes and Donaldson 1969). Atlantic cod, however, are able to increase their freezing resistance by producing antifreeze glycoproteins when exposed to low temperatures. These lower the plasma freezing point noncolligatively and allow cod to survive ice contact at water temperatures ranging from -1.1 to -1.6°C depending on the age and/or size of the fish (Hew et al. 1981; Fletcher et al. 1982, 1987; Kao and Fletcher 1988; Goddard et al. 1992). The developmental stage at which cod become able to synthesize antifreeze is not known.

Cod eggs and larvae may be exposed to subzero temperatures and ice contact for extended periods of time while drifting with the Labrador current. The present study was carried out to determine the freeze resistance of cod eggs and larvae, and thus elucidate their capacity for survival under such environmental conditions.

Materials and Methods

Adult cod (>50 cm) were obtained from an experimental aquaculture facility in Bay Bulls, Newfoundland, in January 1991. These fish were initially caught in bays around the Avalon peninsula, Newfoundland, during the 1990 summer trap fishery and were subsequently maintained in sea cages.

Approximately 40 cod were transported to the Ocean Sciences Centre, Logy Bay, Newfoundland, and held under seasonally ambient conditions of temperature and photoperiod in a 6000-L tank supplied with seawater pumped directly from the ocean. Water temperature in the tank was recorded several times a week using a Cole-Palmer "Quick" thermometer (model 8517-00) accurate to 0.1°C . Cod were fed capelin twice a week.

Mating behaviour was first observed in the tank on March 3 1991, and eggs were found on the surface of the water 2 d later (ambient water temperature 0.2°C). An egg collector was attached to the tank outflow and was checked daily for newly spawned, fertilized eggs. Batches of eggs, generally at the two- to eight-cell stage, were transferred to incubation containers within 24 h of spawning. Experiments were carried out between March and June 1991.

Eggs were incubated in rectangular plastic baskets (approximately 5L) with 400- μm -mesh sides in a flow-through system, semisubmerged in a wet bench. They were held under seasonally ambient conditions of temperature and photoperiod. Near-surface water temperatures were measured daily in each egg basket (see Fig. 2). Each morning, dead eggs were syphoned from the bottom, and the water surface was sprayed with a mixture of 100 mg streptomycin sulphate/L and 60 mg penicillin-G/L (Sigma) dissolved in filtered seawater to control bacterial growth. Within 4 d of hatching, larvae were offered live rotifers (*Brachionus plicatilis*); later larval stages were fed *Artemia salina* nauplii.

Freezing Points of Egg and Larval Fluids

Perivitelline fluid was collected by applying a glass capillary tube to a small incision made in the chorion of eggs at the two- to eight-cell stage (40 eggs per capillary tube), and the samples so obtained were frozen under mineral oil until analyzed. Whole-egg fluid was collected from 0.5-mL batches of eggs at the two- to eight-cell stage. Eggs were briefly blotted dry on Whatman No. 4 filter paper and homogenized in 1.5-mL Eppendorf tubes. Homogenates were centrifuged for 2 min at 8000g and the supernatant was removed from the cellular debris and frozen until analyzed.

Yolk-sac and feeding-stage larvae were briefly rinsed once in 50% seawater, twice in distilled water, and larval fluids obtained by homogenizing 0.5-mL volumes of rinsed larvae in 1.5-mL Eppendorf tubes. Homogenates were centrifuged for 10 min at 4000g and the supernatants frozen until analyzed. Two samples of feeding-stage larvae (0.6 mL of larvae per sample) were washed, homogenized in 0.5 mL of distilled water, and centrifuged as above. The resulting supernatants were lyophilized, stored frozen, and redissolved in a minimum of distilled water immediately prior to analysis. The freezing and melting temperatures of egg and larval fluids were determined using a Clifton nanoliter osmometer (Clifton Technical Physics, Hartford, New York). Using this technique, the freezing and melting behaviour of an ice crystal within the sample is observed under a microscope. The difference between the melting and freezing points of the crystal is termed thermal hysteresis and is a direct measure of antifreeze activity (Kao et al. 1986).

Freeze Resistance of Cod Eggs and Larvae

Initial experiments were carried out by adding batches of 25–50 eggs or larvae to a beaker of seawater (500 mL) at 0°C in a Neslab (Endocal) refrigerated bath. Over a period of 2 h, the temperature was gradually lowered to between -1.7 and -1.8°C , where it was held for a further 24 h. In an attempt to ensure that ice crystals were distributed throughout the water column, ice was added both to the surface of the water and to the bottom of the beaker in association with 1-mm-diameter glass beads frozen in seawater. An air stone was used to ensure uniform water temperature in the beaker and to keep the ice crystals, eggs, and larvae constantly moving.

None of these experiments resulted in egg or larval mortalities. However, as it was not certain that the eggs or larvae had come into contact with ice under these experimental conditions, further experiments were carried out in which direct contact between the egg or larval surface and ice could be ensured.

The observation chamber used in experiments where eggs or larvae were brought into contact with ice under controlled temperature conditions is shown in Fig. 1. It was constructed entirely of glass and consisted of an inner chamber (3.5-mL volume) encased in a cooling jacket. The cooling jacket (external length and diameter 8 and 4 cm, respectively) was connected to a Neslab (Endocal) recirculating temperature control unit that pumped 50% aqueous isopropanol coolant through the jacket via insulated tubing. Access to the inner chamber from the outside was through two ports, both 1.0 cm in diameter, as indicated in Fig. 1. Using this apparatus, the temperature of the inner chamber could be regulated to within 0.02°C . Inner chamber temperatures were monitored using a thermistor probe (Cole Parmer model LN4192 702A) attached to a digital thermometer (Cole Parmer model 8502-25) and inserted through and sealed into the lower port (Fig. 1). Responses of eggs or larvae to freezing temperatures within the chamber were observed using a dissecting microscope.

Experiments on cod eggs were carried out in a glass sample vial (0.7-mL volume, 6-mm external diameter) inserted through and sealed into the inner chamber via the upper port (Fig. 1A). Batches of eggs were placed in the vial along with a volume of seawater or mineral oil sufficient to submerge the eggs. The remainder of the inner chamber was filled with a saturated NaCl solution to prevent freezing at very low temperatures. The temperature of the eggs within the vial was measured using an implantation thermistor (YSI Series 5000, Cole Palmer) (Fig. 1A).

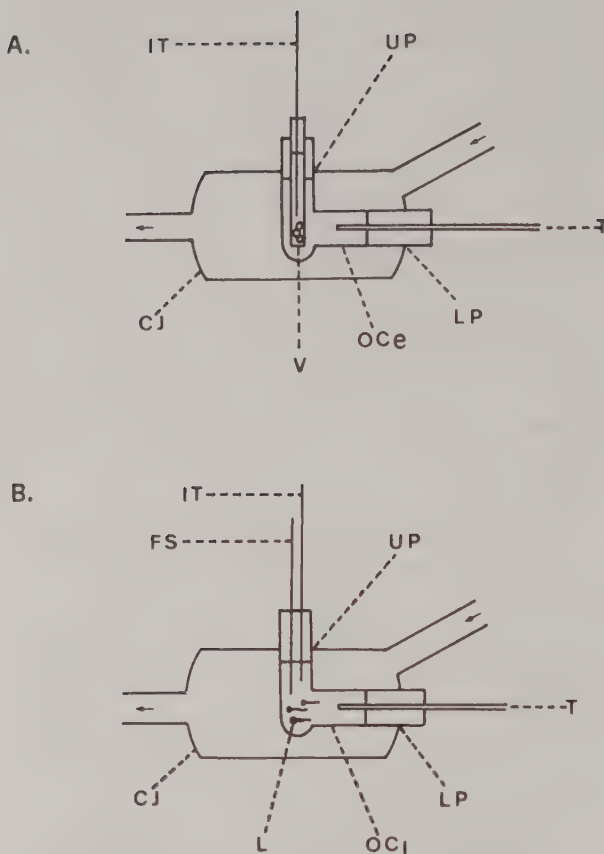


FIG. 1. Cooling apparatus for (A) egg and (B) larval freezing experiments. IT = implantation thermistor; CJ = cooling jacket; UP = upper port; LP = lower port; T = thermistor; V = glass vial containing eggs in oil or seawater; OCe = inner glass observation chamber containing a saturated NaCl solution; L = larvae in seawater; FS = frozen wooden splint; OCI = inner glass observation chamber containing seawater. Arrows show the direction of coolant (50% aqueous isopropanol) flow.

Three variables that might affect egg freezing at temperatures below the freezing point of the egg interior are the presence of ice crystals in the sea, the ability of ice crystals to propagate through the chorion and initiate nucleation and freezing of the egg interior, and wave action (mechanical disturbance). The rationale for the third variable is that when biological fluids are undercooled (i.e. cooled below their freezing temperatures without solidifying), internal nucleation and freezing can be induced by mechanical disturbance alone.

Using the above apparatus, four sets of experiments were carried out to determine the freezing temperatures of intact, newly fertilized eggs (two- to eight-cell stage) under conditions designed to test the importance of the above variables. Each experiment was carried out in duplicate using 11–47 eggs per experimental run. In all cases, the eggs were added to the vial at 0°C and the temperature was lowered at a steady rate of 0.17°C per minute until it reached –17°C, the lowest temperature possible for the apparatus. The experiments were as follows:

(1) undisturbed eggs in frozen seawater, (2) mechanically disturbed eggs in frozen seawater, (3) undisturbed eggs in oil in the absence of ice, and (4) mechanically disturbed eggs in oil in the absence of ice. In experiments 1 and 2, ice crystals

were added to initiate freezing of the surrounding seawater when the temperature of the eggs had declined to –1.8°C. In experiments 3 and 4, eggs were blotted dry on filter paper and immersed in mineral oil. The absence of seawater excluded the possibility of ice being formed on the outside of the egg and propagating through the chorion.

Experiments 2 and 4 were carried out to determine if a mechanical disturbance could cause the eggs to freeze. This was done by either stirring the eggs with the thermistor probe (oil) or vigorously tapping the bench (ice). Comparisons among experiments 1, 2, 3, and 4 allowed conclusions to be drawn as to whether mechanical disturbance and the presence of external ice have any effect on egg freezing temperatures and whether egg freezing is due to ice propagation across the chorion or internal nucleation of egg fluids.

The importance of the chorion in protecting the egg contents from freezing was examined using the observation chamber apparatus (Fig. 1A). Eggs in the late embryo stage were nicked under a dissecting microscope using a pointed scalpel blade. When an effective incision had been made in the chorion, a drop of perivitelline fluid could be seen escaping from the egg. Groups of 10 nicked eggs were placed in seawater in the vial and the temperature was lowered as described above. Eggs with nicked chorions were found to freeze at temperatures higher than the freezing point of seawater. Thus, once the temperature had decreased to approximately –0.9°C, ice crystals were brought into contact with the eggs using a wooden splint that had been soaked in seawater and then frozen. This experiment was repeated seven times with both nicked and intact eggs at the same stage of development.

All of the above experiments were of relatively short duration (<2 h); therefore, in four additional experiments, early blastula stage eggs were exposed, for 21.5 h, to the following freezing conditions: (1) seawater at –2°C, (2) seawater at –20°C, (3) oil at –2°C, and (4) oil at –20°C. These experiments were carried out using groups of eggs either placed in seawater or blotted dry and suspended in mineral oil and sealed in screw cap vials. The vials were, in turn, sealed into small glass jars filled with a saturated NaCl solution, thus eliminating any possibility of ice crystals propagating into the vials of eggs from an external source. The glass jars were then held at –20°C in a freezer, or at –2°C in a Neslab constant-temperature bath. All jars were undisturbed.

The freezing temperatures of free-swimming larvae were determined directly in the inner glass observation chamber filled with seawater (Fig. 1B), the glass vial used for the egg freezing experiments having been previously removed. Larvae (three to five per experiment) and ice were placed in the inner observation chamber containing seawater at –0.7°C. The upper port was closed with a stopper through which were inserted the implantation thermistor probe and a frozen seawater-soaked wooden splint for maneuvering ice. The temperature within the observation chamber was reduced in steps of 0.01–0.02°C and could be held steady to within 0.01°C. At selected temperature steps, the larvae were brought into contact with ice using the frozen splint. To test whether mechanical disturbance (touching), rather than ice propagation into the larvae, was responsible for causing nucleation and freezing of their undercooled tissue fluids, larvae were cooled below their freezing point (as above) and touched with an unfrozen wooden splint in the absence of ice.

To determine their response to prolonged undercooling, larvae (5 d posthatch) were held for several days in beakers of ice-free seawater at –1.75°C in a Neslab (Endocal) refriger-

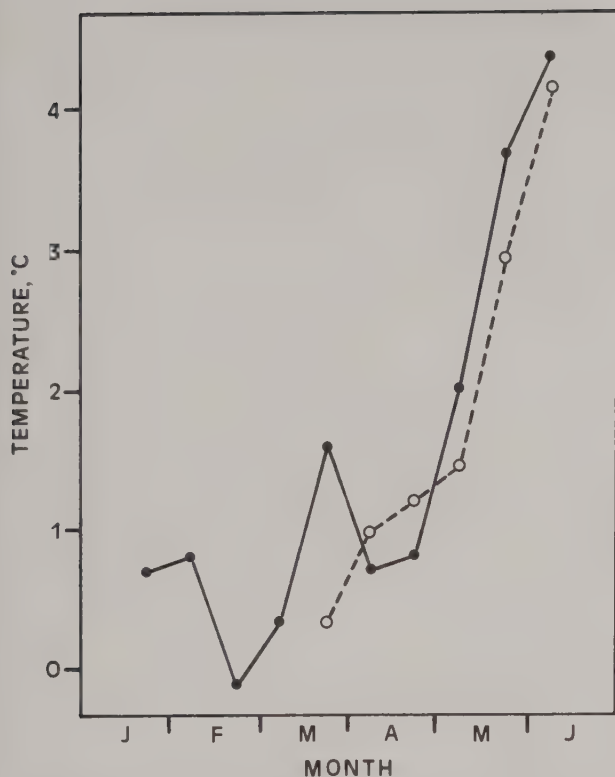


FIG. 2. Broodstock and egg incubation temperatures. ● = water temperature experienced by brood stock cod before and during the spawning period. All values are semimonthly means of temperatures recorded several times each week. Eggs were first observed on the water surface on March 5, 1991. ○ = water temperature experienced by the developing eggs and larvae used in all experiments. All values are semimonthly means of temperatures recorded daily.

ated bath. Undercooled larvae were not fed during these experiments.

Results

The water temperatures experienced by the adult brood stock and by the developing eggs and larvae used in these experiments are presented in Fig. 2.

Freezing Points of Egg and Larval Fluids

Homogenates of cod eggs at the two- to eight-cell stage (up to 24 h after spawning) froze at $-0.78 \pm 0.02^\circ\text{C}$ (Table 1). No evidence of thermal hysteresis or antifreeze activity was detected in any of the egg homogenates or perivitelline fluid samples. None of the larval homogenates examined contained any detectable antifreeze (Table 1). Even when the supernatant from homogenized feeding stage larvae was concentrated threefold by freeze-drying, antifreeze activity could not be detected. All egg and larval fluids froze at temperatures approximately 0.7 – 1.0°C higher than the freezing point of seawater (Table 1).

Freezing Resistance of Cod Eggs

Initial experiments with cod eggs were designed to simulate extreme oceanic conditions: low temperatures and ice present. Eggs were incubated for 24 h in beakers of aerated, icy sea-

water at temperatures between -1.7 and -1.8°C . The eggs proved to be very resistant to freezing, as none of them froze, even when the seawater in the beakers became solid.

In the observation chamber (Fig. 1A), frozen eggs appeared opaque and unfrozen eggs transparent when a light source was placed behind them. Each condition could be readily identified. When the eggs were cooled in seawater or oil in the absence of any mechanical disturbance, freezing did not occur until the temperature had decreased to approximately -10 to -11°C (Table 2). Most of the undisturbed eggs had not frozen by the time the temperature had declined to -17°C . At these very low temperatures, the aqueous isopropanol solution in the cooling jacket surrounding the observation chamber usually turned icy, making it difficult to count exact numbers of eggs as they froze. However, in one experimental run of undisturbed eggs in seawater the eggs could be clearly seen, revealing that only 13% of the eggs had frozen when the temperature reached its minimum value of -17°C . These experiments showed that cod eggs can be exposed to temperatures well below any they would experience in nature without spontaneously freezing.

The results of the experiments carried out in oil indicate that at temperatures below -4.0°C , the freezing process can be initiated internally in the absence of external ice. Mechanically disturbing the eggs during the cooling process (both in oil and seawater) initiated freezing at between -4 and -7°C , and all the eggs in these experiments were frozen by the time the temperature had declined to -8.5°C (Table 2). Thus, at temperatures below -4.0°C , eggs are undercooled to the point where they are in danger of freezing, whether external ice is present or not.

Longer term experiments demonstrated that eggs incubated in oil or seawater did not freeze when exposed to -2.0°C for 21.5 h (Table 3). Exposing these eggs to -2.0°C for a further 38 h (total time 59.5 h) did not result in any of them freezing. However, after 21.5 h at -20°C , all the eggs in seawater and 79.4% of the eggs in oil had frozen (Table 3).

Eggs (late embryo stage) in which the chorion had been nicked froze at temperatures between -1.32 and -1.48°C when cooled in contact with ice ($n = 70$). Mean temperature between groups at which the first egg froze was $-1.38^\circ\text{C} \pm 0.01$ (number of groups = 7). Control groups of eggs at the same stage of development remained unfrozen at -5.0°C .

Freezing Resistance of Cod Larvae

Newly hatched or feeding-stage larvae appeared remarkably resistant to freezing when cooled in beakers of aerated seawater with seed ice present. They could be observed swimming near the ice and only froze when the seawater neared or reached its freezing point and ice crystals had built up throughout the beaker.

Larvae used in the contact freezing experiments displayed a typical set of responses when they froze: they stuck to the inoculating ice, convulsed for a few seconds, froze completely, and turned opaque. Yolk-sac larvae (sampled within 24 h of hatching) and feeding-stage larvae (>20 d old) had mean freezing points of $-1.34 \pm 0.01^\circ\text{C}$ and $-1.36 \pm 0.02^\circ\text{C}$, respectively, which were not significantly different using a *t*-test. Results of these experiments are summarized in Table 4. Larvae that were cooled below their freezing point and touched with the wooden splint in the absence of seed ice crystals did not freeze, indicating that in these experiments, larval freezing was a direct result of inoculating the integument with ice.

The behavioural responses of cod larvae to low temperatures in the absence of ice are summarized in Table 5. Larvae (5 d

TABLE 1. Freezing temperatures, melting temperatures, and thermal hysteresis ($^{\circ}\text{C}$) of cod egg and larval fluids. Each whole-egg homogenate sample consisted of 40–50 eggs. Each perivitelline fluid sample consisted of fluid pooled from 40 eggs. Numbers of sample groups examined are given in parentheses. Larval homogenate values presented are from one 0.5-mL volume of yolk-sac larvae. Values are presented as means $\pm$ 1 SE.

Sample type	Freezing point	Melting point	Thermal hysteresis	Date of experiment
Whole-egg homogenate	-0.78 ± 0.02 (4)	-0.77 ± 0.02 (4)	0.01 ± 0.002 (4)	1–15 May 1991
Perivitelline fluid	-0.98 ± 0.07 (7)	-0.95 ± 0.08 (7)	0.03 ± 0.02 (7)	1–15 May 1991
Larval homogenate	-0.88	-0.83	0.05	23 April 1991
Seawater	-1.76 ± 0.02 (2)	-1.76 ± 0.03 (2)	0	1 May 1991

TABLE 2. Freezing temperatures of intact cod eggs. N = total number of eggs observed. Temperatures at which the first egg was observed to freeze (freezing initiated) and at which the last egg froze (100% eggs frozen) were noted for each experiment. The majority of undisturbed eggs remained unfrozen during these experiments (lowest experimental temperature = -17°C approximately).

Exp.	Incubation conditions	Treatment	N	Freezing initiated ($^{\circ}\text{C}$)	100% eggs frozen ($^{\circ}\text{C}$)
1	Seawater	Undisturbed	58	-11.6	—
2	Seawater	Disturbed	54	-7.0	-8.5
3	Oil	Undisturbed	73	-10.7	—
4	Oil	Disturbed	81	-4.1	-8.5

TABLE 3. Freezing behaviour of early blastula stage cod eggs after 21.5 h at low temperature. N = number of groups (11–98 cod eggs per group). Percentage frozen values are the means of all group percentages $\pm$ 1 SE.

Treatment	N	Percentage frozen
Seawater at -2.0°C	8	0.00 ± 0.00
Seawater at -20.0°C	6	100.00 ± 0.00
Oil at -2.0°C	7	0.00 ± 0.00
Oil at -20.0°C	5	79.40 ± 8.75

after hatching) remained alive for at least 48 h when held at -1.75°C in the absence of ice. Shining a bright light on the larvae, or touching them, could initiate a swimming response, although they were inactive unless these stimuli were used. No larvae were observed to freeze when undercooled to -1.75°C , and normal behaviour was resumed when the larvae were returned to 0.0°C .

Discussion

Our results clearly indicate that cod eggs and larvae are remarkably resistant to freezing at subzero temperatures in the

presence of ice. None of the eggs examined froze at temperatures above -4.0°C , while cod larvae froze at approximately -1.35°C when they came in direct contact with ice.

Strategies for freeze resistance that could be used by planktonic organisms with minimal swimming ability, such as cod eggs and larvae, include (1) having body fluids isosmotic with the surrounding seawater, (2) producing antifreeze proteins to lower the freezing point of the extracellular fluids, and (3) possessing external tissues that are impermeable to the inward propagation of ice, coupled with the ability of the tissue fluids to undercool without freezing spontaneously.

Strategies 1 and 2 can be discounted for cod eggs and larvae. Egg perivitelline fluid froze at -0.98°C , egg homogenates at -0.78°C , and larval homogenates at -0.88°C , while seawater freezes at approximately -1.8°C (depending on salinity). The induction of antifreeze production in adult and juvenile cod is temperature dependent (Fletcher et al. 1987; Goddard et al. 1992). It is not known whether cod eggs or larvae are capable of producing antifreeze glycoproteins, and if they are, at what temperature. The temperatures experienced by our fish (Fig. 2) may have been low enough to induce antifreeze production. Indeed, the brood stock cod had plasma thermal hysteresis levels of $0.15 \pm 0.02^{\circ}\text{C}$ in February ($n = 9$) (unpublished

TABLE 4. Contact freezing temperatures ($^{\circ}\text{C}$) of live yolk-sac and feeding-stage larvae of cod. Freezing points (FP) are given as means $\pm$ 1 SE. Values of n are given in parentheses. Max FP and Min FP are the overall maximum and minimum freezing points recorded for an individual larva. There was no significant difference (t -test) between the mean freezing points of yolk-sac and feeding-stage larval cod.

Stage	Dates of experiments	Mean FP	Max FP	Min FP
Yolk-sac larvae 0–1 d posthatch	23–29 May 1991	-1.34 ± 0.01 (30)	-1.19	-1.43
Feeding larvae >20 d posthatch	6–14 May 1991	-1.36 ± 0.02 (30)	-1.10	-1.65

TABLE 5. Larval responses to cooling in the absence of ice.

Temperature (°C)	Larval response
0.00 to -1.36	Larvae occasionally swim and appear active
-1.36 to -1.56	Swimming reduced. Larvae occasionally convulse without freezing
-1.56 to -1.75	Activity completely reduced, but larvae respond when touched or subjected to bright light
0.00	On returning to 0.00, larvae resume normal activity levels (observed for 24 h postrevival)

data). However no antifreeze was found in any of the egg or larval homogenates examined.

From our experiments, it seems likely that strategy 3 is used by both the eggs and larvae to avoid freezing. In the absence of ice, eggs and larvae survived undercooling down to the freezing point of seawater. Neither showed any tendency to spontaneously nucleate and freeze even when mechanically disturbed at these low temperatures. Thus, it appears that, in the short term at least, egg and larval tissue fluids are stable when undercooled to -1.8°C . In addition, at temperatures below -1.3°C , larval swimming activity was much reduced, and below -1.56°C , it ceased completely. This latter observation has two main implications for cod larvae. Risk of freezing due to internal nucleation might increase when larvae are exposed to very low temperatures for extended periods of time. Lack of swimming activity at temperatures below -1.56°C might reduce the risk of spontaneous nucleation and thus increase the chances of larval survival. However, cessation of swimming could also result in a change of position in the water column. The buoyancy of larval cod can vary with time from hatch, larval condition, temperature, and salinity (density of the medium) (Neilson et al. 1986; Yin and Blaxter 1987). Larvae are found most commonly in the top 30 m of the water column. It seems likely that healthy larvae are neutrally buoyant at some point within this depth range and that in the short term, cessation of swimming activity would not have a significant effect on their position in the water column with respect to temperature. However, longer exposure could result in changes in feeding, condition, and, therefore, buoyancy. The consequences of this in terms of survival are unknown, but would seem to be negative.

Much of the freezing resistance of cod eggs is attributable to the chorion. Indeed, the fact that freezing temperatures were essentially the same whether eggs were cooled in oil or frozen seawater suggests that the chorion can completely block ice propagation down to temperatures of -10 to -17°C . Eggs in which the chorion had been nicked lost most of their protection, with their freezing temperatures resembling those of cod larvae rather than intact eggs.

Resistance to freezing seems to be a feature of eggs of teleosts from northern latitudes. Eggs from a population of capelin (*Mallotus villosus*) that spawns intertidally at Balsfjord, northern Norway, have been found to survive for many hours in frozen films of seawater at temperatures down to -5.0°C , while eggs of benthic spawning capelin populations are equally cold tolerant (Davenport and Stene 1986; Davenport 1989). Aarset and Jorgensen (1988) reported that the pelagic eggs of plaice (*Pleuronectes platessa*) can tolerate ice contact for several hours at -6°C , while the eggs of certain salmonids are also surprisingly tolerant of low temperatures (Harvey and Ashwood-Smith 1982). Eggs of the teleost species used in the above-mentioned

studies develop in diverse habitats, yet all remain unfrozen in the presence of ice at low temperatures. This also seems true of cod eggs. Thus, it can be concluded that cod eggs developing in the waters off the coast of Labrador are in little danger of freezing, provided they have an intact chorion.

The rate of development of cod eggs is temperature dependent (Hardy 1978). Consequently, the colder the sea, the further the eggs will have travelled with the current before larvae emerge from the protection of the chorion. However, once hatched, cod larvae become vulnerable to freezing when exposed to the oceanic conditions that can occur along the northeast coast of Newfoundland during the spring (see Table 6, particularly minimum temperature values).

The results of the present study suggest that the temperature at which larvae can freeze (approximately -1.35°C) is 0.5°C lower than the freezing point of their body fluids. This suggests that ice propagation into the larvae is retarded by a barrier of some kind, the most likely candidate for which is the external epithelium.

A recent study revealed that ice propagation temperatures across the skin of two marine species (winter flounder, *Pleuronectes americanus* (formerly *Pseudopleuronectes americanus*), and ocean pout, *Macrozoarces americanus*) are approximately 1.1°C lower than the plasma freezing points (Valerio et al. 1992). Thus, fish skin appears to present a considerable barrier to ice propagation. In general, however, fish freeze at temperatures within approximately 0.1°C of their plasma freezing points (DeVries and Lin 1977; Fletcher et al. 1986, 1988). Therefore, ice must enter the fish at a site offering less resistance to ice propagation than the skin. One likely site is the gill lamellar surface where only a thin layer of epithelial cells separates the blood in the lamellar capillary sheet from the environment (Morrison 1988).

The gills of yolk-sac and early feeding stage larvae consist solely of gill arches devoid of filaments, and the distance between the gill blood vessels and the exterior is greater than the thickness of the larval skin. At an incubation temperature of 7°C , gill filaments take at least 2 wk to begin development, while secondary lamellae do not appear until 5 wk posthatch (Hunt von Herbing and Boutilier 1990; C. M. Morrison, DFO, Halifax Laboratory, Halifax, N.S., pers. comm.). Up until this point, cod larvae rely on cutaneous, rather than branchial respiration; thus, the gills do not function as they do in post-metamorphic cod. The cod larvae used in the present study were incubated at relatively low temperatures (0 – 3°C). Thus, their gills were probably elementary in structure, as described above. The simple structure of early larval gills could make them a more effective barrier to ice propagation than those of the adults and thus explain why larvae freeze at lower temperatures than their body fluids do.

Given that fully developed gills are likely to be the weakest barrier to ice propagation, slow rates of larval development at subzero temperatures would be advantageous for larval cod. The longer it takes for the gills to become fully functional, the greater the likelihood that the larvae will have drifted into ice-free water.

The structure of the skin, slow development of sensitive gill structures, and a reduction in swimming activity may all help to increase the chances of cod larvae surviving at low temperatures. However, the fact remains that should cod larvae contact ice at temperatures below -1.3 to -1.4°C , this may well prove lethal.

In the northwest Atlantic, ice cover can be extensive and sea temperatures low (down to -1.8°C under the ice and in the

TABLE 6. Temperature at the surface and 10, 20, and 30 m depth at Station 27 (47°31'50"N, 52°35'10"W). Temperatures (°C) are given as means $\pm$ 1 SE of individual values (number of values in parentheses) recorded at varying intervals each month from 1950 to 1990 at Station 27. The maximum and minimum temperatures recorded at each depth over this period are given.

Month	Surface	10 m	20 m	30 m
March	-1.08 $\pm$ 0.07 (71) max 0.30, min -1.76	-1.24 $\pm$ 0.07 (61) max 0.14, min -1.79	-1.26 $\pm$ 0.07 (57) max 0.14, min -1.78	-1.24 $\pm$ 0.07 (62) max 0.05, min -1.80
April	-0.17 $\pm$ 0.08 (84) max 2.26, min -1.66	-0.39 $\pm$ 0.08 (68) max 1.35, min -1.67	-0.60 $\pm$ 0.07 (76) max 0.59, min -1.67	-0.61 $\pm$ 0.07 (85) max 1.49, min -1.74
May	1.78 $\pm$ 0.12 (133) max 5.08, min -1.20	1.25 $\pm$ 0.10 (134) max 5.00, min -1.44	1.00 $\pm$ 0.09 (148) max 4.51, min -1.66	0.44 $\pm$ 0.07 (161) max 3.47, min -1.62

upper water layers), at the time of year when cod eggs and larvae are known to be present in the plankton (Symonds 1986; Fissel et al. 1989; Fissel and Tang 1991). It appears that within any one geographical location, there is very little interannual variation in the timing of the spawning event and that low surface temperatures will not significantly postpone cod spawning (Serebryakov 1967; Ellertsen et al. 1989). Cod larvae have been found in the plankton off Labrador in April and May when temperatures in the surface waters are uniformly below zero (Serebryakov 1967; Postolaky 1968). Even as far south as Station 27, a sampling station located approximately 10 km east of St. John's, Newfoundland, and considered to be representative of the inshore Labrador current (Drinkwater and Trites 1991), April temperatures in the surface layers can be below -1.65°C, well below the mean larval freezing point of -1.35°C (see Table 6).

Thus, in unusually cold years when ice cover persists off the coast of Newfoundland and Labrador into the late spring and early summer, the risk of larval mortality directly attributable to freezing may be significant.

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Estimates of Phosphorus and Nitrogen Cycling by Fish Using a Bioenergetics Approach

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Kraft, C. E. 1992. Estimates of phosphorus and nitrogen cycling by fish using a bioenergetics approach. *Can. J. Fish. Aquat. Sci.* 49: 2596–2604.

A modelling approach based on bioenergetics was used to estimate the role of fish in nutrient recycling. Phosphorus excretion (P_U) and nitrogen excretion by young-of-the-year (YOY) and older yellow perch (*Perca flavescens*) were estimated for a limnetic system: Lake Memphremagog. This model successfully predicted P_U by comparison with previous estimates for yellow perch. YOY fish contributed more to limnetic nutrient cycling through excretion than older age-classes, and YOY fish could also serve as an important P sink relative to algal sinking losses. Volumetric P_U by YOY yellow perch reached a maximum of $0.3 \mu\text{g P}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$; N excretion peaked at $10 \mu\text{g N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. The ratio of excreted N:P varied fourfold during the summer. P_U estimates were also sensitive to changes in fish P content, for which few values have been reported for early life stages.

Une méthode de modélisation basée sur la bioénergétique a été utilisée pour estimer le rôle des poissons dans le recyclage des éléments nutritifs. L'excrétion de phosphore (P_U) et d'azote par des perchaudes (*Perca flavescens*) jeunes de l'année et plus âgées a été estimée pour un système limnétique : le Lac Memphremagog. Ce modèle a réussi à prévoir le P_U par rapport à des estimations précédentes pour la perchaude. Les poissons jeunes de l'année contribuent davantage au cycle limnétique des éléments nutritifs par l'excrétion que les poissons plus âgés et pourraient également servir d'important puits à P par rapport aux pertes algaires. Le P_U volumétrique des perchaudes jeunes de l'année a atteint un maximum de $0,3 \mu\text{g P}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$; l'excrétion de N a atteint un sommet de $10 \mu\text{g N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. Le ratio N:P excrété a varié dans une proportion de quatre fois durant l'été. Les estimations de P_U étaient sensibles aux changements de la teneur en P des poissons, sur laquelle on dispose de peu de données aux stades de vie précoces.

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The contribution of fish egestion and excretion to nutrient cycling in lakes has seldom been included in models of nutrient cycling and algal production, despite great interest in (1) the effects of fish on phytoplankton and (2) the sources of nutrients for phytoplankton. Early versions of food web models incorporating biotic nutrient recycling and algal production in lakes included zooplankton excretion and ignored the potential contribution of fish (e.g. Scavia 1979; Carpenter and Kitchell 1987; Scavia et al. 1988), reinforcing the assumption that fish nutrient regeneration is negligible by comparison with zooplankton.

The potential for a significant contribution by fish to phosphorus (P) recycling in limnetic systems via excretion was first raised by Kitchell et al. (1975); who concluded that P excretion by bluegill (*Lepomis macrochirus*) in a small lake may support algal populations during periods of low P availability. Lamarra (1975) also concluded that egestion and excretion by common carp (*Cyprinus carpio*) could provide a substantial proportion of the total P loading in a small lake.

A subsequent study by Nakashima and Leggett (1980b) rejected the hypothesis that P excretion by fish is a major nutrient source for primary producers, based on work with adult yellow perch (*Perca flavescens*). They estimated that, by comparison with zooplankton, fish contributed only a small fraction to the internal recycling of P in the epilimnion of a large lake during summer (Nakashima and Leggett 1980b). Yet a recent estimate of all P inputs to the pelagic system of a small lake indicated that P derived from inshore feeding by fishes was a

large flux, comparable with inputs from physical-chemical fluxes (Carpenter et al. 1992). Brabrand et al. (1990) similarly estimated that seasonal P release by roach (*Rutilus rutilus*) equaled P loading from the watershed: midsummer P loading by fish was twice the external P supply. Boers et al. (1991) found similar rates of P excretion by fish and zooplankton in a shallow lake.

Experiments examining the impact of fish on algal production in enclosures have also generated interest in this subject. Threlkeld (1987) conducted mesocosm experiments that indicated that nutrient release by living and dead fish might play an important role in P cycling and algal production. Reintersen et al. (1986) concluded that the patchy release of P by fish produced differences in algal community structure in enclosures. Vanni and Findlay (1990) suggested that differences in phytoplankton abundance and community structure in a mesocosm experiment could be due to the direct contribution of fish excretion, as well as zooplankton excretion, to P cycling.

Studies demonstrating the impact of variable N:P excretion by zooplankton upon algal communities (Elser et al. 1988; Sterner 1990) also raise questions concerning N:P excretion rates by fish. In this paper, I present a modeling approach to estimate the potential contribution of fish to nutrient recycling. Mass-balance comparisons of the potential for nutrient intake and elimination by fish can be made by using knowledge about the energetics of fish growth, in combination with information on the nutrient content of prey and fish tissue. This approach is similar to that proposed by Olsen et al. (1986) to estimate P release by zooplankton using a mass-balance equation:

$$(1) P_r = P_i - P_g$$

where the flux of released P (P_r) is given by the difference between what is ingested (P_i) and what is used for growth and reproduction (P_g). The simplicity of this approach, as well as the difficulty of experimentally measuring P egestion and excretion rates (Durbin 1976; Ruben and Bennett 1981), makes this an attractive alternative.

Methods

Estimating Nutrient Excretion

P excretion by fish was estimated according to the mass balance

$$(2) P_U = P_C - P_G - P_F$$

where P_U is P excreted (urinary), P_C is P consumed, P_G is P allocated to growth, and P_F is P lost through egestion (fecal). This mass balance can be implemented provided estimates are available for consumption, growth, P content of predator and prey, and the P assimilation efficiency. If a fish lost weight, P_G was set equal to zero. Nitrogen (N) excretion was estimated according to the same mass balance by substituting analogous terms for N instead of P.

P_C can be calculated from estimates of prey consumption and prey P content. Prey consumption for adult yellow perch was estimated using a bioenergetics model developed by Kitchell et al. (1977), as modified by Hewett and Johnson (1987) for incorporating variable caloric density of predators and prey. This bioenergetics model uses a mass balance based on calories to estimate consumption for an individual fish, given a known growth and temperature history for each individual, as well as information on the caloric content of both predator and prey. Prey consumption for young-of-the-year (YOY) yellow perch was estimated using the Kitchell et al. (1977) model, as modified by Post (1990). To make the units in Post (1990) consistent with those required by Hewett and Johnson (1987), RA was set equal to 0.06481 and ACT was set equal to 4.4.

The fish community of the limnetic system chosen for analysis, the southern basin of Lake Memphremagog (Vermont/Quebec), was dominated by yellow perch during the years modeled (Nakashima and Leggett 1980b); hence the only estimated fish contribution to nutrient cycling was that by yellow perch. This lake was chosen for analysis because of the thorough data sets available in the published literature, as well as previous attempts to estimate fish contributions to P cycling in that lake (Nakashima and Leggett 1975, 1978, 1980a, 1980b). All estimates assume that nutrients excreted by yellow perch are mixed throughout the lake's south basin, which has a volume of $301 \times 10^6 \text{ m}^3$ and mean depth of 6.9 m (Nakashima and Leggett 1975).

Nakashima and Leggett (1980b) provided monthly growth rates (fork length) for 0+ to 5+ yellow perch in Lake Memphremagog during 1972–73. These lengths were converted to wet weight (grams) using a fork length – total length regression from Nakashima and Leggett (1975) and a total length – wet weight regression for Green Bay yellow perch (Willis and Guy 1989) (Tables 1 and 2). For comparison with Nakashima and Leggett's (1980b) estimates of P_C and P_U for three yellow perch length intervals, model estimates of P_C and P_U for different yellow perch age-groups were apportioned among length intervals as indicated in table 2 from Nakashima and Leggett (1980b).

Different temperature regimes were used to model nutrient cycling by YOY and older yellow perch. These temperatures

TABLE 1. Growth intervals and population estimates used to estimate nutrient cycling by 0+ and 1+ yellow perch. Weights and 0+ and 1+ yellow perch population estimates were calculated from data in Nakashima and Leggett (1980b) as described in the text.

Age	Date	Population size	Weight (g)
0+	June 15	3.11×10^9	0.08
0+	Aug. 1	3.46×10^8	1.7
0+	Oct. 15	2.79×10^7	2.8
1+	May 1	1.7×10^6	2.9
1+	Sept. 1	5.2×10^5	13.9
2+	Sept. 1	9.4×10^4	38.9

TABLE 2. Growth intervals used to estimate nutrient cycling by 101–150, 151–200, and >200 mm Lake Memphremagog yellow perch. Weights were calculated as described in the text. Yellow perch of different ages were apportioned among length intervals as indicated in table 2 from Nakashima and Leggett (1980b).

Age	Month	Weight (g)
2+	June	23.2
	July	27.9
	Aug.	34.5
	Sept.	38.9
	Oct.	40.3
3+	June	47.7
	July	59.2
	Aug.	73.8
	Sept.	80.3
	Oct.	84.6
4+	June	88.7
	July	98.6
	Aug.	117.4
	Sept.	122.4
	Oct.	123.1
5+	June	137.6
	July	148.0
	Aug.	162.0
	Sept.	163.9
	Oct.	165.0

were taken from Lake Memphremagog monthly mean water temperatures (Nakashima and Leggett 1980b).

YOY and adult yellow perch were assumed to eat a diet of zooplankton that remained constant in energy and P content. YOY yellow perch ate only zooplankton; adult yellow perch ate a combination of zooplankton and fish prey, similar to proportions presented in Nakashima and Leggett (1978). Estimated energy densities for yellow perch and their prey were $550 \text{ cal} \cdot \text{g}^{-1}$ (wet weight) ($1 \text{ cal} = 4.1868 \text{ J}$) for zooplankton prey and $1200 \text{ cal} \cdot \text{g}^{-1}$ for 1+ and older yellow perch and ranged from 800 to $1000 \text{ cal} \cdot \text{g}^{-1}$ for YOY yellow perch growing from June 15 to July 15 and $1000 \text{ cal} \cdot \text{g}^{-1}$ for the remainder of the summer (Tarby 1977; Treasurer 1989).

Adult yellow perch and fish prey P content was estimated as 2% of dry weight (McQueen et al. 1992) with a dry to wet weight ratio of 0.25 (Kelso 1973; Jezierska 1974; Tarby 1977). A N content of 0.10 as a proportion of dry weight was assumed for YOY and adult yellow perch, based on estimates for 16- to 30-g fish (Jezierska 1974; Davis and Boyd 1978). Zooplankton prey were assumed to be 0.1 dry weight as a proportion of wet weight (Dabrowski and Rusiecki 1983; Reinertsen et al. 1990), with a P content of 0.01 as a proportion of dry weight (Nakashima and Leggett 1980a) and a N content of 0.1 as a proportion of dry weight (Andersen and Hessen 1991).

Little information was available on the P content of larval fish. For initial model estimates, I assumed that P varied linearly as a proportion of wet weight from 0.003 to 0.005 from day 15 to 45 (by assuming that P content was a constant 0.02 as a proportion of dry weight and that dry weight as a proportion of wet weight varied linearly from 0.15 to 0.25). This will be referred to as the "Mid P" estimate. Two alternative estimates of larval fish P used in model simulations were derived in the following manner:

(1) "Low P": P varied linearly as a proportion of wet weight from 0.0015 to 0.005 from day 15 to 45. I assumed that P content varied linearly from 0.01 to 0.02 as a proportion of dry weight during this period. Dry weight as a proportion of wet weight varied linearly from 0.15 to 0.25.

(2) "High P": P was a constant 0.005 as a proportion of wet weight from day 15 to 45. I assumed that P content was a constant 0.02 as a proportion of dry weight. Dry weight was a constant 0.25 as a proportion of wet weight.

Absorption efficiency of total P for adult and YOY yellow perch was estimated as 72%, based on laboratory estimates for adult yellow perch (Nakashima and Leggett 1980a). Absorption efficiency of total N was estimated as 80% (Brett and Groves 1979). Therefore, $P_F = 0.28 \times P_C$ and $N_F = 0.20 \times N_C$.

Population Estimates

The 0+ yellow perch population size in the southern basin of Lake Memphremagog was backcalculated from an estimate of 2+ and older yellow perch (Nakashima and Leggett 1980b) and data from tables 2 and 6 in Nakashima and Leggett (1980b) (Table 1). The lowest September population estimate for 101- to 150-mm yellow perch was used as a starting point from which to backcalculate yellow perch population abundance at earlier ages, since this size range corresponded to the total 2+ age population (Nakashima and Leggett 1980b). Mortality rates were calculated from population estimates for Oneida Lake. Nielsen (1980) provided the following yellow perch population estimates for a 10-yr period: 0+ (October), 1+ (May, September), and 2+ (September). Mills and Forney (1989) provided YOY yellow perch population estimates over a 25-yr period for 18-mm yellow perch (no date given) and 0+ fish on August 1 and October 15. Average mortality rates were calculated from these data, assuming an exponential mortality rate. Tarby (1977) reported that yellow perch fry in Oneida Lake were 20 mm on June 16; Mills and Forney (1989) reported that YOY yellow perch in Oneida Lake reached 18 mm from mid-to late June. The earliest mortality rate was calculated for a period extending from June 15 to August 1, which provided a conservative estimate of population size. Model runs for YOY yellow perch began on June 15 (Table 1).

Results

YOY yellow perch P_U , expressed on a volumetric basis, was nearly an order of magnitude greater than P_U by adult yellow perch (Table 3). Model estimates of maximum volumetric P_U by the adult (2+ and older) yellow perch population were similar to estimates by Nakashima and Leggett (1980b). Daily ration estimates derived from the model show a good correspondence with published field estimates (Fig. 1). Model P_C estimates correspond well with previous estimates for the two smaller size-classes of yellow perch, but the model underestimated P_C for the largest fish compared with Nakashima and Leggett's (1980b) estimates (Fig. 2). Some model P_U estimates vary by as much as a factor of 2 from previous estimates by

TABLE 3. Estimated maximum volumetric P excretion (P_U) for fish in Lake Memphremagog.

Origin P_U (data source)	Estimated P_U ($\mu\text{g P} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$)
YOY yellow perch (this study)	0.52
2+ and older yellow perch (this study)	0.06
Littoral zone fishes (Nakashima and Leggett 1980b)	0.18
2+ and older yellow perch (Nakashima and Leggett 1980b)	0.08

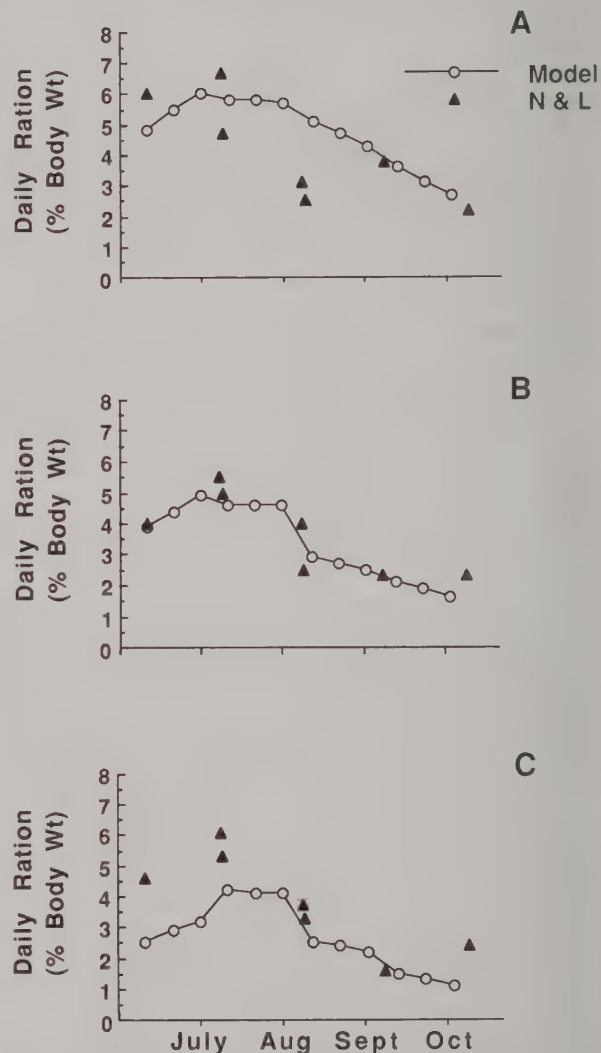


FIG. 1. Seasonal daily ration estimates for yellow perch as estimated by Nakashima and Leggett (1978) (labeled "N & L") and bioenergetics model estimates (labeled "Model") determined by using growth and temperature data from the same lake. (A) 101-150 mm fork length (ages 2+ and 3+); (B) 151-200 mm fork length (ages 3+ and 4+); (C) >200 mm fork length (ages 4+ and 5+).

Nakashima and Leggett (1980b) (Fig. 3). Model estimates are lower earlier in the season for the smallest size-class, higher through much of the season for the middle size-class, and always lower for the largest size-class. Model estimates of individual P_U by YOY yellow perch in August and September were

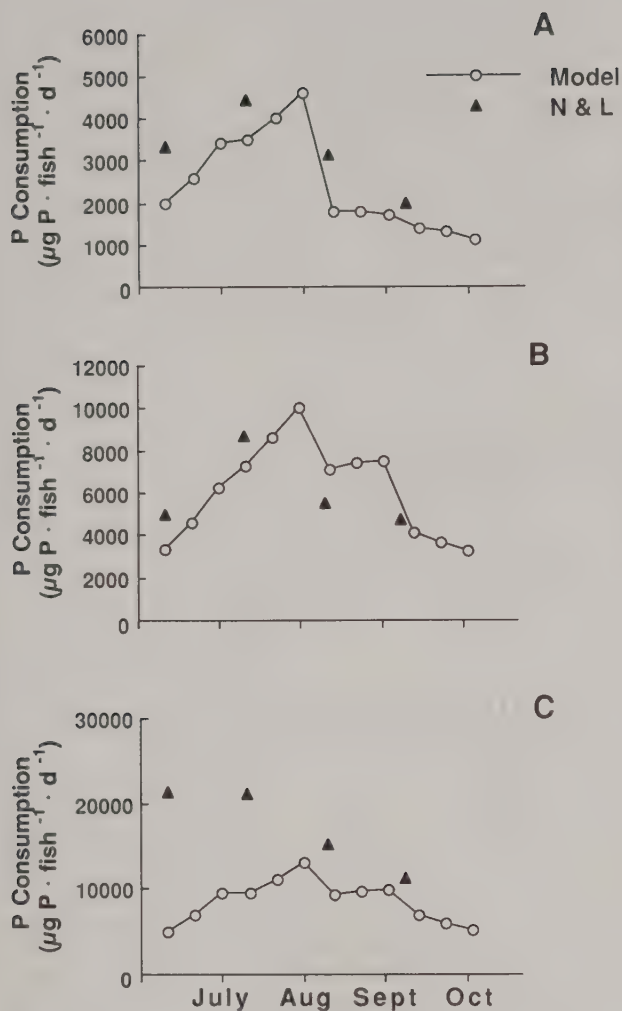


Fig. 2. Seasonal P consumption (P_C) estimates for an average individual yellow perch from Lake Memphremagog. Model estimates (labeled "Model") were made using the methods described in text and are compared with previous published estimates (labeled "N & L") for the same fish population (Nakashima and Leggett 1980b). (A) 101–150 mm fork length (ages 2+ and 3+); (B) 151–200 mm fork length (ages 3+ and 4+); (C) >200 mm fork length (ages 4+ and 5+).

170 and 250 $\mu\text{g P}\cdot\text{d}^{-1}$, respectively, by comparison with estimates by Nakashima and Leggett (1980b) of 80 and 77 $\mu\text{g P}\cdot\text{d}^{-1}$.

Volumetric P_U by YOY yellow perch reached a maximum of 0.3 $\mu\text{g P}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ at 37 d after hatching (Fig. 4A), declined sharply and remained lower for a 17-d period, and then increased back to previous levels before beginning a steady decline. Volumetric N excretion (N_U) by YOY fish (Fig. 4B) ranged from 0 to 10 $\mu\text{g N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$, peaking around day 30 and then declining throughout the remainder of the summer. The ratio of N:P excretion by YOY yellow perch declined through the summer (Fig. 4C) but varied fourfold. The amount of P incorporated in YOY yellow perch production (Fig. 4D) peaked at nearly 1.0 $\mu\text{g P}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ around day 30 and then declined through the summer. This quantity (P_G) represents the P sink present in the fish population. Seasonal variation in the amount of YOY yellow perch biomass is shown in Fig. 4E, as

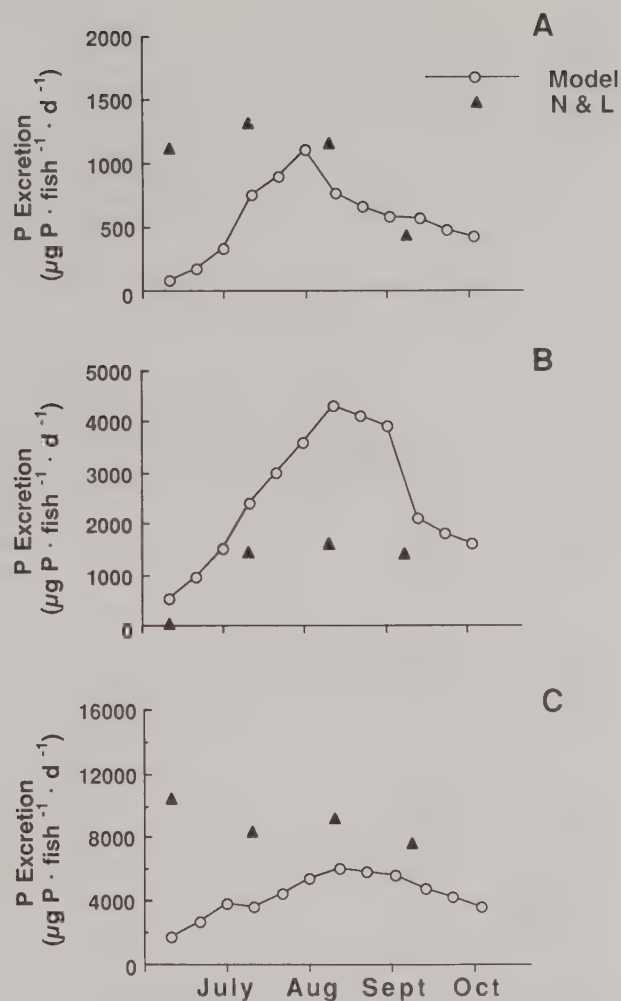


Fig. 3. Seasonal P excretion (P_U) estimates for an average individual yellow perch from Lake Memphremagog. Model estimates (labeled "Model") were made using the methods described in text and are compared with previous published estimates (labeled "N & L") for the same fish population (Nakashima and Leggett 1980b). (A) 101–150 mm fork length (ages 2+ and 3+); (B) 151–200 mm fork length (ages 3+ and 4+); (C) >200 mm fork length (ages 4+ and 5+).

estimated from the bioenergetics model based on growth data, population estimates, and other model inputs. The seasonal P budget for an individual fish (Fig. 5) includes daily allocations of P_C to P_G , P_U , and P_F . P_G accounts for a greater proportion of P_C early in the summer; P_U accounts for a greater proportion of P_C later in the year.

The three alternative estimates of P content in fish tissue during the first 45 d posthatch had a major impact on estimates of P_U during this period (Fig. 6A), producing P_U estimates ranging from 0 to 0.5 $\mu\text{g P}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. These different P_U estimates were produced by varying the amount of P incorporated in fish growth, as shown in Fig. 6B.

The sensitivity of P_U to changes in the rate at which P is incorporated in fish production can be seen in Fig. 4A. Large discontinuities in P_U in mid-July and early August were produced by small changes in caloric value and growth rate, which were produced by changes in model input values for these parameters on these dates. A comparison of Fig. 4A and 4D

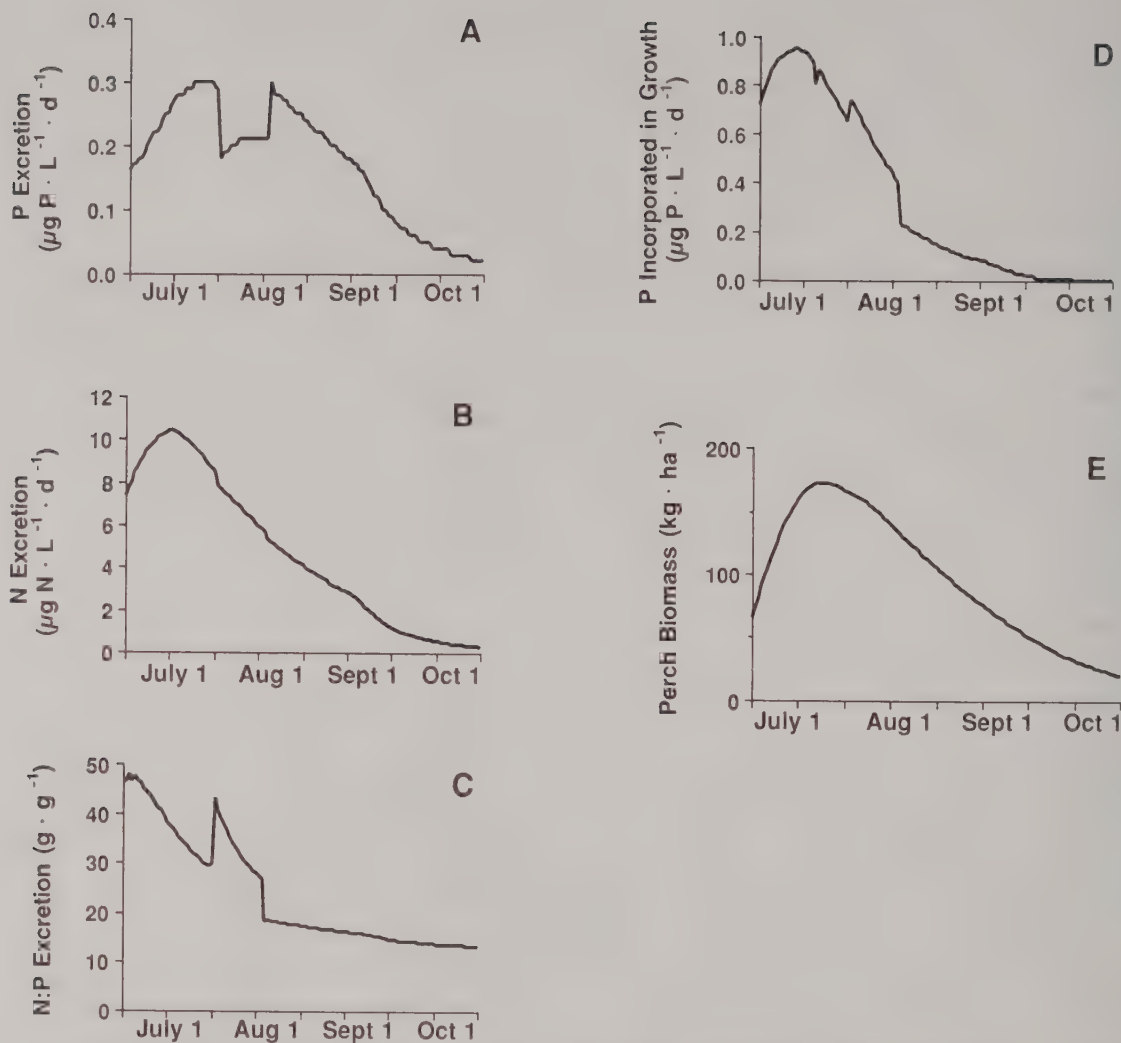


FIG. 4. Estimated components of seasonal nutrient cycling by the YOY yellow perch population in Lake Memphremagog. (A) P excretion (P_U); (B) N excretion (N_U); (C) ratio of excreted N to P; (D) P incorporated in yellow perch production (P_G); (E) estimated seasonal biomass of the YOY yellow perch population (adult yellow perch biomass was negligible by comparison with YOY biomass).

shows that small discontinuities in the rate of P_G produced relatively large changes in the rate of P_U whereas N_U is much less sensitive to changes in growth (Fig. 4B). A 20% increase in N_G produced only a 3% decline in N_U , while a similar increase in P_G produced a 33% decline in P_U . It is unclear whether the rapid changes in P_U produced by the model reflect the potential for highly variable day-to-day changes in P_U or are a model artifact.

N excretion, expressed on a volumetric basis, largely reflects changes in biomass of the YOY population (Fig. 4B). Energy losses due to excretion (losses due largely to the deamination of proteins) are relatively constant throughout the summer. By contrast, P losses through excretion are seasonally variable, depending on the amount of P sequestered by fish production (Fig. 5).

An unexpected observation on the likely role of fish in nutrient cycling is that fish can be a P sink, accounting for a steady P loss from the epilimnion during summer stratification

(Fig. 4D). Different assumptions about the P content of larval fish did not alter the conclusion that P sequestered in growth could represent an important P sink.

Discussion

Comparison with Previous P Excretion Estimates

A major result of the model estimates presented here is that YOY yellow perch P excretion is an order of magnitude greater than that by older age-classes (Table 3). Since all volumetric estimates presented in this paper assume that yellow perch excretion is distributed evenly throughout the water column, these estimates are conservative. Aggregations of YOY yellow perch in the top 1 m of the water column, physical factors that retain P_U in the epilimnion, and daily inshore/offshore movements (Post and McQueen 1988) could further increase volumetric P_U . Nakashima and Leggett (1980b) similarly

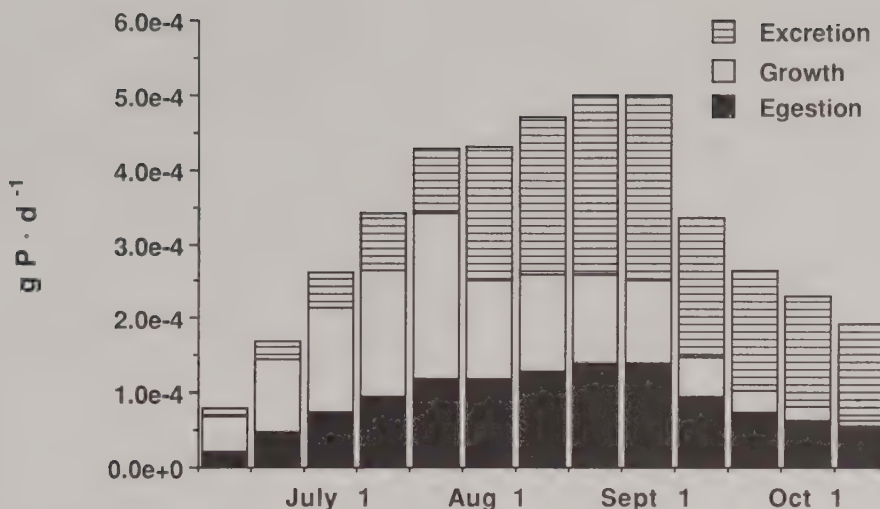


FIG. 5. P budget for an individual YOY yellow perch, showing the amount of daily P consumption allocated to growth, excretion, and egestion throughout the growing season.

observed that higher densities of fishes in the littoral zone increased estimated volumetric P_U (Table 3).

The observation that nutrient regeneration by YOY yellow perch may be an order of magnitude greater than that produced by older age-classes is consistent with observations from Oneida Lake that YOY yellow perch biomass exceeds the biomass of age 3+ fish and that YOY yellow perch production exceeds production by 3+ and older fish during most years (Mills et al. 1987). A similar observation of the importance of younger fish has been made for larval and YOY alewife (*Alosa pseudoharengus*) in Lake Michigan, which account for 50% of the annual zooplankton consumption by alewife in that lake (Hewett and Stewart 1989). The disproportionate impact by YOY fish is a product of both biomass and metabolic allometry (Post 1990).

Volumetric YOY P_U rates produced by the model were still an order of magnitude lower than estimates of zooplankton P regeneration estimated by Nakashima and Leggett (1980b) using regressions from Peters (1975). Brabrand et al. (1990) also found that roach excretion rates were less than 1/10 of those from zooplankton as calculated by the equations of Peters (1975). However, Olsen and Østgaard (1985) observed that zooplankton P excretion rates calculated with Peters' (1975) equations may overestimate nutrient release rates by an order of magnitude due to underestimates of C:P ratios in phytoplankton food sources. Lehman and Naumoski (1985) observed a similar reduction in *Daphnia* excretion rates for animals fed P-deficient algae. Carpenter et al. (1992) estimated that the summer average P excretion by fishes in a northern temperate lake was comparable with excretion by zooplankton under two different food web configurations.

Since daily ration estimates are the basis for estimating fish excretion using equation 2, comparisons between field estimates and model estimates of consumption (Fig. 1) are critical to the success of this approach. Nakashima and Leggett (1980b) used field estimates of prey consumption (Nakashima and Leggett 1978) to estimate P_C and then subsequently used a mass-balance approach identical to that presented in this paper to estimate P_U , using laboratory estimates of P assimilation

efficiency to estimate P_F and growth information to estimate P_G . For all three size-classes, the bioenergetics model predicted the same trend and range of values as Nakashima and Leggett's (1978) estimates of consumption.

Model estimates showed that individual P_C peaked in early August (Fig. 2), but was similar at the start and end of the growing season due to weight increases that compensated for higher weight-specific consumption rates (daily ration) in June than in October. Nakashima and Leggett's (1980b) higher estimates of P_C for the largest size-class could have been due to larger daily ration estimates or differences in the following assumptions: (1) I used constant crustacean prey P content of 0.01 and fish prey P content of 0.02 as a proportion of dry weight; Nakashima and Leggett (1980a) reported values for cladocerans and copepods ranging from 0.007 to 0.011 and fish values from 0.017 to 0.031. (2) I estimated crustacean prey dry weight as 10% of wet weight and fish prey dry weight as 25% of wet weight; Nakashima and Leggett (1980b) reported daily ration in terms of wet weight and prey P content as a proportion of dry weight, but did not state the proportions used to convert dry weight estimates to wet weight; and (3) my estimates of fish weight, derived from a total length – wet weight regression for Green Bay yellow perch could have differed from weights used by Nakashima and Leggett (1980b).

Model estimates showed that daily individual P_U rose to a late summer peak for all three size-classes. Differences in P_U estimates (Fig. 3) between Nakashima and Leggett (1980b) and this study were magnified by differences in estimated P_C , but could also have been partly due to different assumptions about the rate at which P was incorporated in fish production. Nakashima and Leggett (1980b) used a regression of fork length versus fish P content to estimate the amount of P sequestered due to growth; I assumed that P content was a constant proportion of fish weight. The Nakashima and Leggett (1980b) fork length – total P regression and a length-weight relationship for Green Bay yellow perch produced estimates of P content ranging from 0.4 to 1.1% of fish wet weight at fork lengths ranging from 49 to 230 mm. Nakashima and Leggett's (1980b) P content estimates for fish greater in length than 89 mm were

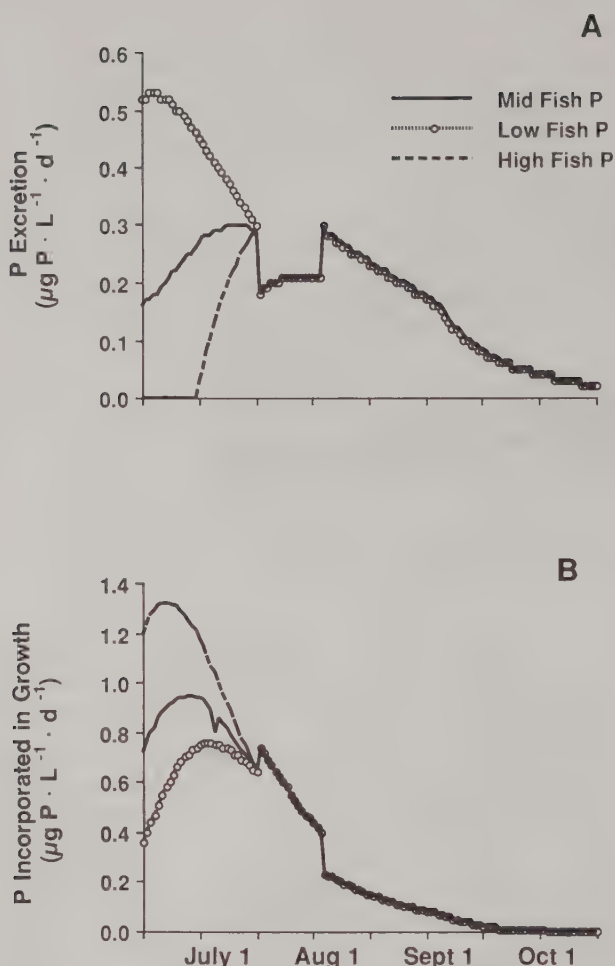


FIG. 6. Influence of three different assumptions about the proportion of P in fish tissue during the first 45 d posthatch on (A) estimated seasonal P excretion and (B) estimated seasonal amount of P incorporated in YOY yellow perch production. Assumptions for "Mid Fish P," "Low Fish P," and "High Fish P" estimates are described in the text.

greater than my P content estimates. At lengths greater than 150 mm, their estimated P content was greater than 5% of fish dry weight, which is greater than most values reported for any fish species (Davis and Boyd 1978). Such differences in estimated P content were important because small relative differences in P_G produced large relative differences in estimated P_U (Fig. 4A and 4D).

P_U estimates are very sensitive to changes in estimates of the P content of fish (Fig. 6A), for which few values have been reported for early life stages. A rare published study of P content for larval fish (McCay et al. 1936) reported the P content of brook trout (*Salvelinus fontinalis*) eggs as 0.23% of wet weight, sac fry as 0.29%, fry as 0.20%, and fingerlings as 0.13–0.37%. Further estimates of the P content of larval fish will be necessary to accurately estimate P_U for larval stages.

The P contents of postlarval fish have been more widely reported than for larvae. Fish for which P contents have been published include 17 warmwater and coolwater species that had P levels ranging from 1.95 to 5.22% of dry weight (Davis and Boyd 1978). Many estimates have been made as part of

aquaculture studies, but less is known about the range of variation in natural systems.

Fish as a Nutrient Sink

Estimated summer total P biomass in seston has been estimated as 10–25 $\mu\text{g P} \cdot \text{L}^{-1}$ for the south basin of Lake Memphremagog (Nakashima and Leggett 1980b). Assuming an algal sinking loss rate of $0.03 \cdot \text{d}^{-1}$ (Carpenter 1992), the estimated sinking loss from sedimentation was $0.3\text{--}0.75 \mu\text{g P} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$, which is in the same range as estimated P loss rates due to P incorporated in YOY fish biomass. The estimated sinking loss rate was calculated from Stoke's Law for an algal community composed of green and blue-green algae (Carpenter 1992). This estimate is conservative, since it was calculated for an epilimnetic depth of 3 m, less than the mixed layer depth for the south basin of Lake Memphremagog. Total P biomass generally remains steady during the summer in Lake Memphremagog (Nakashima and Leggett 1980b), which indicates that epilimnetic summer P losses are compensated by external P inputs captured through algal production. Interannual changes in the P sink represented by fish could influence interannual differences in summer average algal biomass reported by Nakashima and Leggett (1980b). Different assumptions about the P content of larval fish did not alter the conclusion that P sequestered in growth could represent an important P sink relative to algal sinking losses.

Fish as Regulators of N:P Availability

Fish N excretion estimates in this paper are presented to (1) contrast rates of P_U and N_U , and (2) examine the ratio of excreted N:P.

The bioenergetics model used to estimate consumption accounted for energy losses due to excretion, which allowed N_U to be estimated stoichiometrically by assuming a conversion of $5.94 \text{ cal} \cdot \text{mg NH}_4^{-1}$ (Elliott and Davison 1975). Such stoichiometric N excretion estimates were within 0–15% of values calculated using the mass-balance approach. However, the highly variable nature of P storage and excretion by fish made it impossible to directly estimate P_U through stoichiometric conversions.

Changes in the excreted ratio of N to P (N:P) could affect the relative magnitude of N and P limitation of summer algal growth, an impact of zooplankton suggested by Elser et al. (1988) for three small oligotrophic lakes with little external loading. They observed rapid shifts from N to P limitation of algal growth that coincided with major shifts in zooplankton community composition and speculated that these shifts were produced by variable N and P regeneration rates by zooplankton.

The physiological model of P and N excretion by fish described here is similar to a homeostatic model of zooplankton nutrient excretion developed by Sterner (1990), in which zooplankton adjusted N and P accumulation efficiency to maintain homeostatic N:P in their body mass. I assume that fish maintain a constant molar N:P ratio of 11:1, feed primarily upon zooplankton prey with a constant molar N:P ratio of 23:1, and maintain a N:P absorption efficiency of 1.1:1. Sterner (1990) concluded that mesozooplankton maintain a relatively constant homeostasis of molar N:P around 24:1. Davis and Boyd (1978) estimated mean molar N:P as 6.5:1 for 17 fish species. Due to their higher estimate of P content for most fish, Davis and Boyd (1978) measured lower body mass N:P than the value I used for yellow perch. Higher P contents would accentuate the

potential P sink represented by fish, as well as cause greater seasonal variability in N:P excretion.

A major difference between Sterner's (1990) model and that presented in this paper is that Sterner investigated short-term instantaneous zooplankton excretion dynamics, assuming an unchanging population. Model runs presented here for dynamic fish populations showed seasonal changes in excreted N:P ranging from 47:1 early in the summer to 13:1 later in the growing season. These changes were due to changes in fish growth rates. Higher excreted N:P in early summer was due to rapid daily growth rates, at which time all assimilated P was sequestered as body mass and little P was available for excretion. By contrast, consumed N was available in a quantity greater than body tissue requirements throughout the season. When growth is rapid, the accumulation efficiency, as defined by Sterner (1990), would be greater for P than N in order to maintain homeostatic body mass N:P.

According to the results presented here, zooplanktivorous fish are likely to excrete N:P at levels below or above those found in the zooplankton pool, depending on seasonal fish growth dynamics. Sterner (1990) predicted a direct proportionality between the N:P released by zooplankton and the N:P in the algal pool. The uncoupling of fish N:P excretion from the N:P in the algal pool might increase the potential impact of fish nutrient excretion on algal community dynamics. Fish excretion might reduce algal N:P at times when zooplankton excretion, according to Sterner's model, would further increase algal N:P.

Why Have Fish P Excretion Estimates Been Scarce?

Previous laboratory observations substantiate the modeling approach for estimating P_U outlined in this paper. Fish store P at two- to fivefold greater proportions of body dry and wet weights than their prey, yet the upper limits to this P storage capacity are often reached at typical levels of prey P content (Ketola 1975; Nakashima and Leggett 1980a; Wilson et al. 1982). Nakashima and Leggett (1980a) and Brabrand et al. (1990) also observed that an increase in P_C resulted in greater P_U .

Estimating P_U has sometimes proven difficult in the laboratory (Durbin 1976). Essentially all of the inorganic phosphate that leaves the body of a fish is excreted by the kidneys (Grafflin 1936). Brabrand et al. (1990) found that 85–95% of released P was observed as soluble molybdate-reactive P, with the remainder precipitated as fecal pellets. Ruben and Bennett (1981) observed that stress-related fluctuations of blood calcium levels in fish were produced by dissolution of the skeleton, which also releases P. Longhorn sculpin (*Myoxocephalus octodecemspinosus*) P_U was found to increase following disturbance by handling (Grafflin 1936). Laboratory efforts to estimate fish P_U could be susceptible to experimental conditions inducing stress, making a modeling approach attractive when suitable model inputs are available. A modeling approach also offers the advantage of providing daily estimates of P fluxes.

Fish nutrient excretion estimates can be no more accurate than fish population or biomass estimates. The absence of accurate estimates of total fish biomass in lakes, especially YOY fish, may present the greatest obstacle in evaluating the role of fish in nutrient regeneration. Few comprehensive studies of yellow perch biomass are available for comparison with Oneida Lake biomass and mortality estimates (Chadwick 1976).

Given the strong effect of the biomass dynamics of YOY fish on nutrient excretion, other fish species may have different effects depending on their fecundity and population dynamics

(Carpenter et al. 1992). Several studies reporting minor contributions by fish to overall nutrient recycling have not included estimates of contributions by YOY fish (Nakashima and Leggett 1980b; Boers et al. 1991).

Although many estimates have been made of the role of biota in internal nutrient regeneration during periods of epilimnetic nutrient limitation, fish have received scant attention. What has generally gone unrecognized is the fact that fish can provide access to nutrient pools otherwise not considered as available to epilimnetic algal communities. For example, benthic feeding habits and daily inshore–offshore movements by YOY yellow perch (Post and McQueen 1988) transport nutrients into the pelagic epilimnion. Brabrand et al. (1990) considered P excreted by sediment-feeding roach to supply “new” P to the epilimnion. Carpenter et al. (1992) found that littoral feeding by fish was a major input of P to the pelagic system of a small northern temperate lake under conditions of both piscivore- and planktivore-dominated food webs.

Conclusions

The results presented in this paper suggest that (1) YOY fish play a more important role in limnetic nutrient cycling than older age-classes, (2) the ratio of excreted N:P can vary fourfold during the course of the summer, (3) P_U estimates are sensitive to changes in fish P content, for which few values have been reported for early life stages, (4) YOY fish can be a nutrient sink in limnetic systems, and (5) the modeling approach presented provides a method by which the potential role of fish in nutrient cycling can be evaluated.

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Sigmoid Relationships between Phosphorus, Algal Biomass, and Algal Community Structure

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It has long been recognized that there is a positive relationship between total phytoplankton biomass and eutrophication. Recent independent studies demonstrated that algal biomass (chlorophyll) actually responds in a non-linear, sigmoidal fashion with increasing phosphorus levels among lakes. Chlorophyll has been considered (by some authors) as an inconsistent estimate of algal biomass. Using a wide range of published data, we first demonstrate that the sigmoidal nature of the phosphorus–biomass relationship is quite robust, and not simply generated by a systematic variation in the relationship between algal chlorophyll to cell volume ratio and nutrient levels. We show that the sigmoid relationship with total phosphorus persists whether algal biomass is measured by chlorophyll or biovolume. We hypothesize that this nonlinearity actually reflects an underlying systematic variation in one or more of the components of total phytoplankton biomass. In this paper, we examine two functional size groups and show that the large inedible fraction exhibits a strong, nonlinear response to increasing nutrient levels, while the small edible algae do not vary systematically with phosphorus. We hypothesize that this discontinuous shift in the ratio of edible to inedible phytoplankton should be accompanied by concomitant shifts in the structure of the herbivore community.

On sait depuis longtemps qu'il existe un rapport positif entre la biomasse phytoplanctonique totale et l'eutrophisation. Des études indépendantes récentes confirment que la biomasse algale (chlorophylle) réagit en fait selon une courbe non linéaire sigmoïdale à l'augmentation du taux de phosphore dans les lacs. Certains auteurs considèrent la mesure de la chlorophylle comme étant une évaluation peu cohérente de la biomasse algale. D'après un large éventail de données publiées, nous démontrons d'abord que la nature sigmoïdale de la relation phosphore–biomasse est très marquée et n'est pas simplement le résultat d'une variation systématique dans le rapport entre la chlorophylle algale et le volume cellulaire ainsi que la teneur en éléments nutritifs. Nous démontrons que le rapport sigmoïdal avec le phosphore total subsiste, que la biomasse algale ait été mesurée d'après la chlorophylle ou le volume des bio-éléments. Notre hypothèse est la suivante : cette relation non linéaire reflète en réalité une variation systématique sous-jacente d'un ou de plusieurs éléments de la biomasse phytoplanctonique totale. Dans le présent rapport, nous étudions deux groupes de taille fonctionnels, et montrons que la grande fraction non comestible présente une forte réaction non linéaire à l'augmentation des taux d'éléments nutritifs, tandis que la petite fraction d'algues comestibles ne varie pas de façon systématique par rapport au phosphore. Nous pensons que cette variation discontinue dans le rapport phytoplancton comestible – phytoplancton non comestible doit être accompagnée par des variations concomitantes dans la structure de la communauté herbivore.

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The observation that phytoplankton biomass rises with phosphorus concentrations in lake water has been a staple component of limnological theory for three decades (e.g. Sakamoto 1966; Dillon and Rigler 1974; Jones and Bachmann 1976; Schindler 1977, 1978; Nicholls and Dillon 1978; Smith 1982; Canfield 1983; McCauley et al. 1989). The theoretical idea that algal growth is limited by phosphorus availability has led to the belief that the relationship should be a power function with constant exponent. When power functions of chlorophyll to total phosphorus are fitted for different geographical areas or for different suites of data, exponents are not always equivalent (e.g. Nicholls and Dillon 1978; Janus and Vollenweider 1981; Straskraba 1986; McCauley et al. 1989; Prairie et al. 1989). Recent studies have shown, however, that variation

among lakes in chlorophyll *a* (Chl *a*) with total phosphorus (TP) can be better described by a sigmoidal relationship than by simple linear models (McCauley et al. 1989; Prairie et al. 1989). This observation is significant in terms of lake management and might account for discrepancies among the linear models derived from geographically discreet or relatively small data sets (e.g. Nicholls and Dillon 1978; Paloheimo and Zimmerman 1983; Bierhuizen and Prepas 1985; Dillon et al. 1988). Furthermore, it suggests that explanations for the phosphorus–chlorophyll relationship may be incomplete.

Although the measurement of chlorophyll is one of the most convenient and widely applied methods used to estimate algal biomass, there has been some debate concerning its validity as a measure of phytoplankton biomass (e.g. Tolstoy 1979; Can-

field 1983; Lampou et al. 1982; White et al. 1988). In particular, the relationship between Chl *a* and algal biovolume is thought to be highly variable and generally unpredictable (e.g. Saraceni et al. 1978; Dillon et al. 1988; Aleya and Amblard 1989). This has been attributed to species- or size-specific differences in cell chlorophyll content and/or to the influence of environmental factors, such as light (e.g. Steele and Baird 1965; Ruggiu et al. 1979; Desortova 1981; White et al. 1988; Pridmore et al. 1989) and nutrient levels (e.g. Steele and Baird 1965; Ahlgren 1970; Nicholls and Dillon 1978; Saraceni et al. 1978; White et al. 1988) on the ratio of chlorophyll to cell volume.

The existence of a sigmoid relationship of chlorophyll with TP raises two important possibilities: (1) the relationship between phytoplankton biomass and phosphorus is really linear, but chlorophyll is an inconsistent measure of plant biomass, or (2) chlorophyll is a good measure of plant biomass but our theoretical explanation for the form of the relationship between plant biomass and phosphorus in lakes is overly simplistic. We therefore begin this paper by presenting evidence to show that the curvilinearity in the Chl *a* – TP relationship is not an artefact of the lack of consistency in the chlorophyll to biovolume ratio of cells. We do this by comparing the relationship between algal biovolume and TP among lakes with previous results on the Chl *a* – TP relationship.

The sigmoidal nature of the algal–TP relationship suggests that there are several important features of the nutrient–algal relationship which have yet to be explained. For example, why does the rate of change of algal biomass per change in total phosphorus increase at very low nutrient levels and then decline at very high nutrient levels? Many hypotheses could be advanced to explain both of these features. One explanation might be that the nonlinearities can be accounted for by systematic variation in the biomass of functional algal groups. Another possibility is that systematic variation in average growth or loss rates of algae with TP might yield nonlinear algal–nutrient relationships (McCauley et al. 1989). Much emphasis has been placed recently on how properties of planktonic food webs might influence algal biomass (e.g. Kerfoot and DeMott 1980; Carpenter et al. 1985, 1987; Kerfoot et al. 1985; Dorazio et al. 1987; Kerfoot 1987; Vanni 1987; McCauley et al. 1989; Vanni and Temte 1990; Elser and Goldman 1991) either via direct or indirect interactions, and a further explanation for nonlinear plant biomass – phosphorus relationships might be related to differential responses of algal groups that are either susceptible or insusceptible to grazing by planktonic herbivores. This study tests the hypothesis that nonlinearities in the algal–phosphorus relationship are consistent with changes in growth or loss rates of functional algal groups mediated by shifts in the trophic structure of lakes of differing fertility.

While taxonomic considerations are undeniably important (e.g. Lampert 1981; Infante and Abella 1985), the general categorization of the algal community into functional groups that span taxonomic boundaries is supported by extensive measurements of zooplankton feeding rates (e.g. Burns 1968; Gliwicz 1977; Porter 1977; McCauley and Downing 1985; Hawkins and Lampert 1989) and field manipulations of herbivorous zooplankton (e.g. Porter 1972; Gliwicz 1975; Briand and McCauley 1978; McCauley and Briand 1979; Leibold 1989; Vanni and Temte 1990). Nannoplankton are typically consumed by herbivorous zooplankton, and netplankton are *relatively* insusceptible to grazing (e.g. Porter 1972; Gliwicz 1975; Briand and McCauley 1978; McCauley and Briand 1979; McCauley and

Downing 1985; Leibold 1989). These two groups have been labelled “edible” and “inedible”, respectively. They not only display different short-term responses to manipulation of herbivores, but they appear to respond differently to variation among lakes in TP (Watson and McCauley 1988).

Ecological theory also predicts very different responses for the two groups with enrichment. Classical predator–prey theory (e.g. Rosensweig 1972; Leibold 1989; McCauley et al. 1989) predicts that if the edible group is regulated by herbivores, then enrichment should lead only to an increase in herbivore biomass which subsequently suppresses edible algal density to pre-enrichment levels. Edible algal biomass should therefore be invariant among lakes differing in enrichment. Other predictions are made by theory (Fretwell 1977; Oksanen et al. 1981) that takes into account the potential effects of species at other trophic levels (e.g. carnivorous zooplankton, planktivorous or carnivorous fish). For example, Oksanen et al. (1981) predicted that if productivity of algal prey increases with enrichment, then prey biomass should vary as a step function with increasing lake fertility (e.g. Leibold 1989, fig. 1). The discontinuities in the predicted relationship are produced by the development of additional trophic levels and their subsequent influence on the plant–herbivore interaction.

In this paper, we examine differences in algal community structure among lakes to test whether the sigmoidal pattern observed in the biomass–TP relationship can be accounted for by changes in the relative abundance of edible or inedible phytoplankton.

Data and Analyses

We tested our hypotheses using a wide range of published data on algal biomass, chlorophyll concentrations, and phosphorus concentrations. Mean epilimnetic summer values were used wherever possible, since the inclusion of spring and fall data could affect the relationship between some algal size fractions and TP (e.g. Watson and McCauley 1988). These would also represent values which were close to “equilibrium” values, and eliminate some of the variation around temporal or short-term fluxes. However, some individual observations were also included (16%), although where these were reported over one or more growing seasons for a particular lake, the mean (summer) values were calculated. Where necessary, values were converted to micrograms per litre. For algal biomass, we used only estimates of wet weight or biovolume that had been arrived at using the Utermöhl technique (Vollenweider 1969). Where biovolume was given, it was converted into micrograms wet weight per litre (Vollenweider 1969). A total of 67 different sources of data were used from 362 different lakes over one or more stations or years.¹ Nannoplankton or edible phytoplankton were defined as cells or colonies <35–50 µm in maximum linear dimension, while netplankton or inedible algae were defined as cells or colonies >35–50 µm. The number of observations used to test each hypothesis varied considerably (Table 1) because complete data for algal community structure were not available from all studies.

Following the approach used by McCauley et al. (1989), the shape of each of the curves was examined using two methods: (1) multiple regression analysis (Draper and Smith 1981) with variable selection by backward elimination (Hocking 1976) and

¹A complete set of tabular data is available at a nominal charge from the Depository of Unpublished Data, National Research Council of Canada, Ottawa, Ont. K1A 0S2, Canada.

(2) robust locally weighted sequential smoothing (LOWESS; Cleveland 1979). Logarithmic transformations were used to stabilize residual variability. The multiple regression analysis was used to examine whether relationships were linear or nonlinear by assessing the pattern of residuals and the significance of higher-order terms of the independent variable (i.e. TP). LOWESS is a nonparametric smoothing technique that yields an unbiased estimate of the form of the relationship between two variables, which is not constrained by assumptions about the form of the relationship (Cleveland 1985). It is an especially powerful tool for studying the dependence of y on x when "...the signal is embedded in noise" (Cleveland 1985). Because LOWESS fitting is "model free", it has the flexibility to describe many patterns including those with discontinuous derivatives. LOWESS also includes a robust fitting procedure that "guards against deviant points distorting the smooth points" (Cleveland 1979). Because fitted trends are locally weighted, so-called "outliers" have negligible effects on LOWESS fits. All LOWESS analyses were performed with $\delta = 0$ (the smoothing function considers every single data point), $n - \text{steps} = 2$ (number of iterations), and $f = 0.5$ (weighting factor). The values of $n - \text{steps}$ and f were chosen based on the recommendations of Cleveland (1985).

Results and Discussion

Total algal biomass varies nonlinearly with TP. The parametric analysis and LOWESS fit yield comparable results, showing a sigmoidal relationship between TP and total biomass similar to that exhibited by Chl a (Fig. 1; Table 1). There are, however, slight quantitative differences in the curves derived from parametric and model-free methods. These differences probably arise from constraints inherent in the parametric technique (i.e. the use of polynomial equations to fit the data). Below, we first discuss the LOWESS results and then compare them with the parametric models.

In very oligotrophic systems (i.e. $\text{TP} < 5 \mu\text{g/L}$), increases in TP correspond to very little change in total biomass (Fig. 1A); however, once TP exceeds this level, the rate of increase of algal biomass accelerates dramatically with increasing TP. Over the range of TP from ~ 6 to $60 \mu\text{g/L}$, algal biomass appears to increase at a relatively constant rate, and this rate decreases markedly once TP values surpass $65\text{--}75 \mu\text{g/L}$. The polynomial fit yields a qualitatively similar pattern, but the level of TP at which the rate of change of algal biomass begins to decrease in eutrophic lakes differs substantially from that described by the LOWESS results (Fig. 1A). According to the parametric model, the rate of change in algal biomass begins to decline at $\sim 300\text{--}500 \mu\text{g TP/L}$. In oligotrophic lakes, the results are virtually identical for the two techniques.

It appears, therefore, that the sigmoid relationship between TP and phytoplankton biomass (Chl a) found by McCauley et al. (1989) and Prairie et al. (1989) is not an artefact of those authors' use of chlorophyll to estimate algal biomass. This is demonstrated by the fact that the relationship between algal biomass and TP is shown to be robustly sigmoid when either Chl a or biovolume is used to measure total algal biomass. In fact, the relationship between phytoplankton biovolume and TP is more markedly sigmoidal than that between chlorophyll and TP (Fig. 1B). Over the range of TP where there are the most data ($3\text{--}1000 \mu\text{g/L}$; Fig. 1A), both curves show a similar acceleration phase (Fig. 1B). It is important to note that the nonlinear relationship remains significant if the seeming "outliers" at the extreme ends of the data range are removed and the data anal-

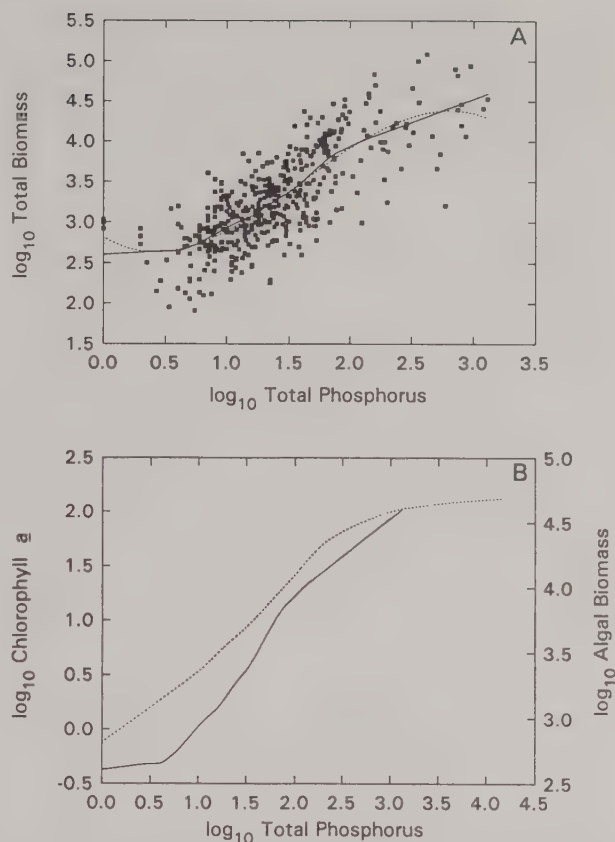


FIG. 1. Relationship between total phosphorus and total algal biomass among lakes. (A) Algal biomass estimated from algal biovolume, showing parametric (dotted curve) and LOWESS (solid curve) fits; (B) LOWESS fits for algal biomass estimated from biovolume (solid curve) and Chl a (dotted curve) (McCauley et al. 1989) versus total phosphorus. All variables in $\mu\text{g/L}$.

ysis is repeated. In addition, variation in the Chl a to biovolume ratio does not account for observed nonlinearities in the relationship between total algal abundance and TP. Inspection of the relationship between the Chl a to biomass ratio and TP shows that this ratio varies in a nonlinear but also nonsystematic fashion with phosphorus (Fig. 2). In fact, the general trend followed by this curve is actually opposite to that which would be expected if the relationship between TP and algal biomass were primarily influenced by this ratio. Neither this, therefore, nor the large amount of residual variation about the curve could account for the sigmoidal nature of the phosphorus–biomass curves. This nonsystematic variation of the Chl a to biovolume ratio with TP may explain some of the discrepancies among studies (e.g. Tolstoy 1979; Dillon et al. 1988) attempting to find a consistent pattern in the relationship using smaller datasets covering a limited range of nutrient levels.

The two algal size categories, designated as edible and inedible algae, respond very differently to variation in TP. Both the parametric and LOWESS analyses show that over the range of TP covered by available data, inedible algal biomass increases nonlinearly with TP by more than 40-fold (Fig. 3; Table 1). Edible algae, on the other hand, do not vary systematically with TP. It appears that the overall shape of the curve of total algal biomass is primarily influenced by changes in

TABLE 1. Partial regression coefficients for the effect of total phosphorus (TP) on $\log_{10}$ total, edible, and inedible algal biomass (estimated from biovolume), found using least squares multiple regression analysis. The number of observations (n), the coefficient of determination (r^2), and the overall F -value are also given, together with the general shape of the corresponding curve obtained using locally weighted robust sequential smoothing (LOWESS; Cleveland 1979) and the range of TP covered by each dataset. Previous results from a similar analysis of the relationship between TP and total biomass estimated as chlorophyll a (Chl a) (McCauley et al. 1989) are included for comparison. TP, algal biomass (biovolume), and Chl a measured in $\mu\text{g/L}$.

Variable ($\log_{10}$)	Parametric model								LOWESS model ^a	TP range
	LTP	(LTP) ²	(LTP) ³	Intercept	n	F	r^2	Prob > F		
Total biomass	-0.92	1.24	-0.26	2.82	451	227	0.60	0.0001	*S	1-1300
Edible biomass	-1.44	1.82	-0.54	2.92	101	9	0.22	0.0001		1-200
Inedible biomass	NS	1.33	-0.46	1.85	101	39	0.44	0.0001	S	1-200
Total Chl a (McCauley et al. 1979)	-0.83	0.34	-0.12	0.03	875	431	0.71	0.0001	*S	1-14093

^aS = sigmoidal, *S = strongly sigmoidal.

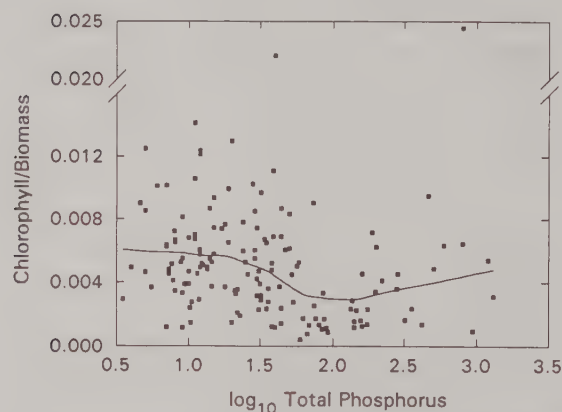


FIG. 2. LOWESS fit of the relationship between total phosphorus ($\mu\text{g/L}$) and Chl a to biomass ratio.

large algae that cannot be ingested by zooplankton rather than by edible phytoplankton.

Under very oligotrophic conditions (i.e. $<5 \mu\text{g/L}$), both inedible and edible algal biomasses are nearly constant or even decline slightly with increasing TP (Fig. 4). From approximately 5 to $30 \mu\text{g TP/L}$, edible algal biomass appears to increase slightly and then levels off, while inedible phytoplankton biomass increases rapidly. There is considerable variation in edible or inedible biomass at a given level of TP, and assessing "significance" of patterns is somewhat problematic. However, the LOWESS analysis shows a discontinuous change over this TP range for both edible and inedible algal biomasses which are measured independently, and the data density is high in the region where most change occurs.

The markedly different responses to increased nutrient levels exhibited by the two algal size fractions seem to be related to their susceptibility to grazing by herbivores. This indicates that the nonlinearity of the total biomass curve may be attributable to factor(s) other than differential responses to nutrient levels. The discontinuities and shifts in community structure are perhaps best illustrated by Fig. 5. Below levels of ~ 8 – $10 \mu\text{g TP/L}$, total biomass is dominated by edible algae. Above this range, there is a transition zone where both the edible and inedible biomasses are similar in relative abundance, but the inedible fraction exhibits a much more pronounced rate of change (Fig. 4). With increasing concentrations of TP (i.e. $\text{TP} > \sim 30 \mu\text{g/L}$) the inedible algae rapidly become more dominant,

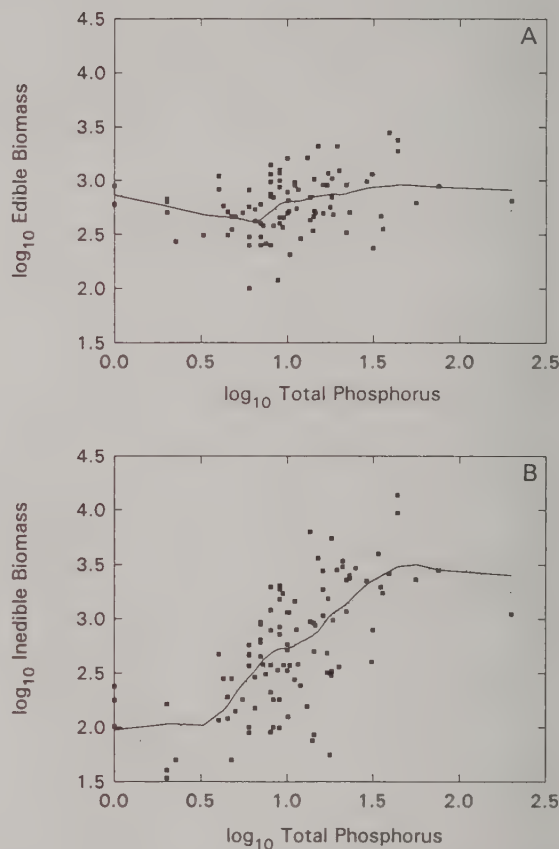


FIG. 3. Relationship between total phosphorus and algal size fractions (biomass; estimated from biovolume), showing parametric (dotted curve) and LOWESS (solid curve) fits. (A) Edible algal biomass; (B) inedible algal biomass. All units in $\mu\text{g/L}$.

until at $\text{TP} > \sim 50 \mu\text{g/L}$ the phytoplankton biomass consists almost entirely of this larger size fraction (Fig. 5).

If the discontinuous change in edible–inedible community structure results from mechanisms proposed by Oksanen et al. (1982), then we should see concomitant changes in the relationship between average herbivore biomass and TP. Specifically, herbivore biomass should increase with TP among lakes with $\text{TP} < 5 \mu\text{g/L}$, and in the region of $5 < \text{TP} < 30 \mu\text{g/L}$, there should be a discontinuous change in herbivore biomass among lakes. Alternatively, the rate of change of zooplankton

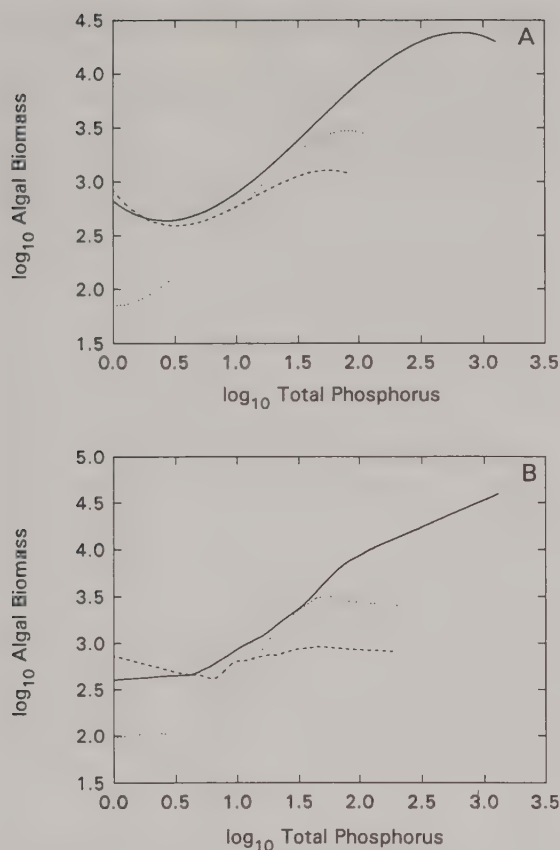


FIG. 4. Relationship between total phosphorus and total (solid curve), edible (broken curve), and inedible (dotted curve) algal biomass. (A) Parametric fits; (B) LOWESS fits. All units in $\mu\text{g/L}$. Note that the calculated intercept for the edible fraction is higher than that of the total biomass simply because the lakes represented in each dataset differ among fractions.

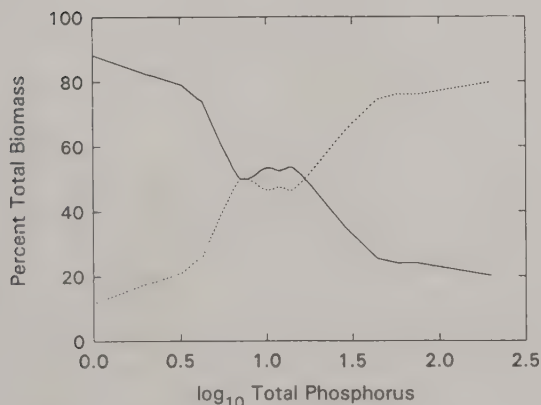


FIG. 5. Relationship between total phosphorus and the relative proportions of edible (solid curve) and inedible (dotted curve) algal biomass. While the curves are obviously mirror images, both have been presented to facilitate interpretation.

biomass could remain the same, and systematic changes in zooplankton community structure could occur. The discontinuous change in edible algae would occur if the herbivorous zooplankton shifted to species with lower attack rates over this range of TP, thereby yielding a higher "equilibrium" concentration of prey (McCauley et al. 1989). Unfortunately, existing studies of among-lake variation in zooplankton biomass (McCauley and Kalff 1981; Hanson and Peters 1984; Pace 1984) have not looked for patterns at this scale of comparison, nor have observations been analysed using model-independent techniques such as LOWESS. We are currently investigating these possibilities.

We recognize that the division of the phytoplankton community into only two functional size groups may limit the potential interpretation of the phosphorus–biomass curve. Analyses based on size and those based on taxonomic divisions may not be mutually exclusive: some of the major taxonomic groups comprise primarily smaller individuals (e.g. Chrysophyta, Cryptophyceae) and others larger algae (e.g. Cyanophyta). Furthermore, as we noted previously, the response of the grazer community to certain dominant taxonomic algal groups could in turn influence the overall shape of the phosphorus–biomass curve. In a subsequent paper, we examine the relationships between major taxonomic groups and phosphorus enrichment and compare them with those of the major size groups that we have described here.

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Models to Predict Potential Occurrence and Density of the Zebra Mussel, *Dreissena polymorpha*

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Limnological features of different lakes may limit the density of, or even completely restrict, populations of the European zebra mussel, *Dreissena polymorpha*. We developed statistical models to predict the occurrence (presence or absence) and density (number per square metre) of *Dreissena* in lakes, based on multivariate correlations between the density of *Dreissena* populations and the limnological characteristics of the lakes they inhabit. We found that both occurrence and average density of *Dreissena* populations were highly correlated with water chemistry. *Dreissena* was not found in lakes with average pH values below 7.3 and concentrations of calcium ion below 28.3 mg·L⁻¹. Above these thresholds, *Dreissena* density was negatively related to concentration of the algal nutrients PO₄ and NO₃.

Certaines caractéristiques limnologiques de différents lacs peuvent limiter la densité, ou même anéantir, des populations de dreissena polymorphe (ou moule zébrée), *Dreissena polymorpha*. En vue de prédire l'occurrence (présence ou absence) et la densité (nombre par mètre carré) de *Dreissena* dans les lacs, nous avons élaboré des modèles statistiques à partir de corrélations à plusieurs variables entre la densité des populations de *Dreissena* et les caractéristiques limnologiques des lacs qu'elles fréquentent. Nous avons observé un degré de corrélation élevé entre les deux facteurs occurrence et densité moyenne et l'hydrochimie. *Dreissena* était absente des lacs dont le pH moyen est inférieur à 7,3 et la concentration en ions calcium inférieure à 28,3 mg·L⁻¹. Au-dessus de ces valeurs seuils, la densité de *Dreissena* était inversement proportionnelle à la concentration des PO₄ et NO₃ contenus dans les éléments nutritifs d'origine algale.

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The European zebra mussel, *Dreissena polymorpha* (Palas), has recently appeared in North America in the Laurentian Great Lakes (Hebert et al. 1989). This mussel can cause changes in freshwater communities and economic damage to man-made structures because of its feeding characteristics, sessile growth habit, and high fecundity (Stańczykowska 1977; Mackie et al. 1989; Griffiths et al. 1991). (Because as yet unnamed, invasive, sibling dreissenid species has been identified (May and Marsden 1992), we will use *Dreissena* in the following study in the broad sense to represent both *D. polymorpha* and the sibling species.)

Useful forecasts of the potential ecological and economic impacts of invading *Dreissena* depend on estimates of both its occurrence (presence or absence) and density (mussels per square metre). Unfortunately, no method currently exists to predict occurrence and density of *Dreissena* in local habitats (Stańczykowska 1977). For example, extrapolation of the success of *Dreissena* in western Lake Erie (our primary North American experience with zebra mussels) to other water bodies can provide only a worst-case scenario. The colonization of western Lake Erie has been unusually successful compared with most European populations. We present statistical models to predict occurrence and density of *Dreissena* in lake habitats. Our models are developed from statistical correlations between *Dreissena* density in European lakes and limnological features of those lakes.

The success of *Dreissena* at colonizing several Great Lakes habitats and the rapidity with which it has spread across Europe do not necessarily indicate that this mussel will invade all North American freshwaters. The European experience shows that *Dreissena* is not found in all water bodies and that among those lakes in which *Dreissena* does occur, population densities can vary by several orders of magnitude. Since *Dreissena* is currently dispersed primarily by humans, some of its zoogeography can be explained by human activity patterns. For example, lakes along shipping channels were the first to be colonized, while *Dreissena* has only recently appeared in headwater lakes in the Alps (Siessegger 1969; Löffler 1979). Dispersal patterns alone, however, cannot fully explain patterns of occurrence. *Dreissena* is sometimes absent from lakes within a district that comprises many connected lakes (e.g. Lundbeck 1926). Population density can vary greatly among those lakes in a district that are colonized.

Low concentrations of dissolved calcium ion are known to affect zoogeographic distributions of several species of freshwater molluscs (e.g. Boycott 1936; Ökland 1983), since calcium is necessary for shell growth and other physiological processes. Stańczykowska (1964) found a weak relationship between calcium concentration and *Dreissena* density in 36 lakes. Sprung (1987) found in laboratory experiments that both fertilization success in eggs and survivorship of *Dreissena* embryos were enhanced by calcium concentrations above 47 mg·L⁻¹ and pH values of about 8.5.

Stańczykowska et al. (1983) and Stańczykowska (1984) found a tendency for *Dreissena* to be absent from highly

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eutrophic lakes that have high levels of phosphorus. Recently, Strayer (1991) found that the zebra mussel in Europe can tolerate such a wide range of climatic temperatures that its predicted zoogeographic distribution in North America may range from northern Canada to near the USA–Mexico border. Strayer (1991) also reported a tendency for European lakes in which *Dreissena* occurs to have higher concentrations of total ions (i.e. harder water).

Dreissena population density may also be affected by a variety of ecological factors. In some studies, fish (Plizska 1956; Biró 1974) and waterfowl (e.g. Borowiec 1983) consumed large amounts of zebra mussels, while in other studies, predation was negligible (Stańczykowska 1990). Negative effects of predation on *Dreissena* populations have yet to be demonstrated. Low food levels would rarely limit *Dreissena* populations in most freshwater environments (Walz 1978a, 1978b; Sprung and Rose 1988).

We searched for statistical relationships between density of *Dreissena* in different lakes and various environmental characteristics of those lakes. We built on previous studies (e.g. Stańczykowska 1964; Strayer 1991) by compiling data for a wide range of lake types and a broad spectrum of environmental variables. In addition, we provide statistical analyses that incorporate quantitative, predictive models.

Methods

Data on European *Dreissena* density and biotic and abiotic environments were collected from a search through over 350 papers published from 1900 to 1990. The literature search produced data for 278 lakes. We plan to make a complete set of our tabular data available in conjunction with a later paper through the Canada Institute for Scientific and Technical Information (Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ont. K1A 0S2, Canada).

Our data set is restricted to European lakes inhabited by zebra mussels (*Dreissena* lakes) or lakes that in our judgement have received potential colonists but lack the mussel. We excluded from the data set any lakes to which *Dreissena* had probably not been introduced (e.g. high Alpine lakes and northern Scandinavian lakes). By excluding lakes that have probably not been colonized, we focused on factors that may determine the survival and growth of *Dreissena* populations in different lakes, exclusive of dispersal patterns that are already known to be strongly influenced by human activity (Stańczykowska 1977). Many lakes without *Dreissena* occurred in lake districts where they were in close proximity to other lakes that did support *Dreissena* (e.g. Lundbeck 1926; Schermer 1931, 1932; Dunn 1954). *Dreissena* also had a high probability of being introduced to lakes of the English Lake District and Scottish Highlands (which all lack *Dreissena*), as these lakes have been heavily used for recreational and commercial purposes; many are connected by locks and shipping canals to waterways inhabited by *Dreissena* (Kerney and Morton 1970; Macan 1970, 1984; Kerney 1974). Heavy boat traffic was the probable cause of the successful invasion of several species of molluscs and aquatic macrophytes into these highland lakes within this century (Macan 1970). Finally, a few lakes without *Dreissena* were located on river systems containing *Dreissena*. For example, although the Goczałkowice Dam Reservoir in Poland lacked *Dreissena*, it received veligers and adults from the Volga River (Krzyżanek et al. 1986).

We also excluded from the data set any lakes to which *Dreissena* had been introduced within the last 50 yr. *Dreissena* den-

sity may fluctuate early in an invasion, and population levels may differ from long-term, equilibrium density (Schlesch 1930; Panyi et al. 1974; Walz 1974). All of the lakes in our data set occur in areas that had either been colonized by *Dreissena* or exposed to *Dreissena* introductions for at least 50 yr (Stańczykowska 1977).

Dreissena population dynamics show two interesting patterns (Stańczykowska et al. 1975; Lewandowski 1982; Ramcharan et al. 1992). First, *Dreissena* densities vary considerably among different lakes. Second, in more than half the lakes with *Dreissena*, mussel density is surprisingly constant from year to year (Stańczykowska et al. 1975; Ramcharan et al. 1992).

We made an effort to collect all published *Dreissena* data for each lake to determine the average density of *Dreissena* in the zone of occurrence. *Dreissena* density often varied among different habitats within a lake and from year to year. We dealt with variability among habitats by averaging as many density estimates as we could find. In some cases, we found only one density estimate for a lake, but these estimates were averaged values of samples that were taken from a range of depths and habitats within each lake. Where sites in a lake were sampled at a range of depths, we defined the zone of occurrence as extending from the shallowest to the deepest depths at which *Dreissena* was found and averaged these values to provide a density estimate. Estimates of density never included mussels smaller than 5 mm. Year-to-year fluctuations in density were also dealt with by averaging all the among-year density estimates that we could find for each lake.

For each lake, we searched the limnological literature for data on morphometry, physical and chemical characteristics, and algal productivity that were collected over the same period as the *Dreissena* data. Morphological variables included lake area, volume, depth, length, width, flushing rate, littoral zone area, and drainage basin area. Physical characteristics included maximum and minimum temperature and oxygen measured at the surface and near bottom, in summer and winter, epilimnion depths, and Secchi transparency. Chemical variables constituted most of the data set and comprised several different measures of phosphorus (total [P], total epilimnetic [P], spring turnover [P], [PO₄], nitrogen (total [N], [NO₂], [NO₃], [NH₃], [NH₄], and water hardness (pH, alkalinity, [HCO₃], [CaCO₃], as well as concentrations of Cl⁻, Mg²⁺, Ca²⁺, K⁺, and Na⁺ (all ion concentrations in milligrams per litre). Measures of phytoplankton were average summer total biomass and algal productivity.

Several limnological variables had natural temporal variation on time scales of hours to years and spatial variation in horizontal and vertical dimensions. We dealt with this variation by averaging all values for each variable. Drainage basin area, lake surface area, and lake volume spanned five orders of magnitude; log₁₀-transformed values for these variables reduced undue influences of large lakes in our statistical analyses.

Values for many of the above limnological variables were lacking for some of the 278 lakes of our original data set. To prepare the final data sets, lakes were eliminated if we could obtain values for less than 50% of the variables. We then prepared two different final data sets by eliminating variables that had missing values and lacked any correlations with either occurrence of *Dreissena* (for the presence-absence data) or density (for the density data). The final data (Tables 1–3) included lakes located in England, Scotland, France, The Netherlands, Denmark, Germany, Switzerland, Austria, Poland,

Hungary, Yugoslavia, Italy, the Commonwealth of Independent States (CIS, formerly the USSR), and Sweden.

Data on water chemistry for different basins of the Laurentian Great Lakes were collected from the literature and averaged using the same techniques as for the European data. In all Great Lakes cases, several values for each variable were averaged to provide a representative estimate for each basin.

We developed three different statistical models: one to predict *Dreissena* occurrence and two others to predict density in the zone of occurrence. All statistical analyses were performed with either SAS (version 6.06.01, SAS Institute), Systat (version 5.0, Systat Inc.), or SPSS-PC+ (version 2.0, SPSS Inc.).

For the occurrence model, we used discriminant function analysis (DFA) on limnological variables to find a statistical function that best distinguished lakes with and without *Dreissena*. We first used a stepwise, parametric DFA to identify variables that were most important in distinguishing the two lake types. This DFA was run on eight lakes lacking *Dreissena* and 16 lakes with *Dreissena* for which we had values for surface area, volume, maximum depth, mean depth, maximum summer surface temperature, minimum summer bottom oxygen, Secchi depth, pH, [Ca], [PO₄], and [NO₃]. The variables pH and [Ca] were identified as most important in separating lakes with and without *Dreissena*. The final DFA model was run on 18 lakes without *Dreissena* and 58 lakes with *Dreissena*. These 76 lakes had records of pH and [Ca]. Error rate of the occurrence model was evaluated by jackknife cross-validation. With this technique the discriminant function is recalculated for each lake, each time deleting only that lake from the data set. The deleted lake is predicted to either have *Dreissena* or not using the model that was developed without use of that lake.

We developed two different models to predict *Dreissena* density. The first was a DFA model, similar to the occurrence model, except that we divided *Dreissena* abundance into three categories: absent, low density, and high density. Lakes with less than 3000 mussels·m⁻² were classified as low density, while those with greater density were classified as high density. The threshold between these two categories (3000 mussels·m⁻²) appeared to represent a natural break in the density frequency distribution (Fig. 1A). This DFA model was also evaluated by jackknife cross-validation.

The second model to predict density was developed by multiple regression using only data for lakes that had *Dreissena*. We first studied the relationships between *Dreissena* density and various water chemistry variables using several different univariate models. We developed the final density model with the variables pH, [PO₄], and [NO₃].

Predictions for the Great Lakes were prepared by using values of pH, [Ca], [PO₄], and [NO₃] for the open waters of different Great Lakes basins in our three final models. We limited our predictions to the Great Lakes for two reasons: (1) these lakes are the areas of immediate concern in terms of the invasion and (2) since *Dreissena* has already had the opportunity to colonize all Great Lakes basins, its patterns of occurrence are a test of our occurrence model.

Results

Latitudinal range was similar for lakes with and without *Dreissena*: lakes with *Dreissena* extended from Sweden to the southern CIS, and lakes potentially colonized by *Dreissena* (because of proximity or watershed) that lacked the mussel extended from Scotland to Yugoslavia.

Dreissena density varied considerably among lakes (Fig. 1A). Most of the limnological variables (Tables 1–3)

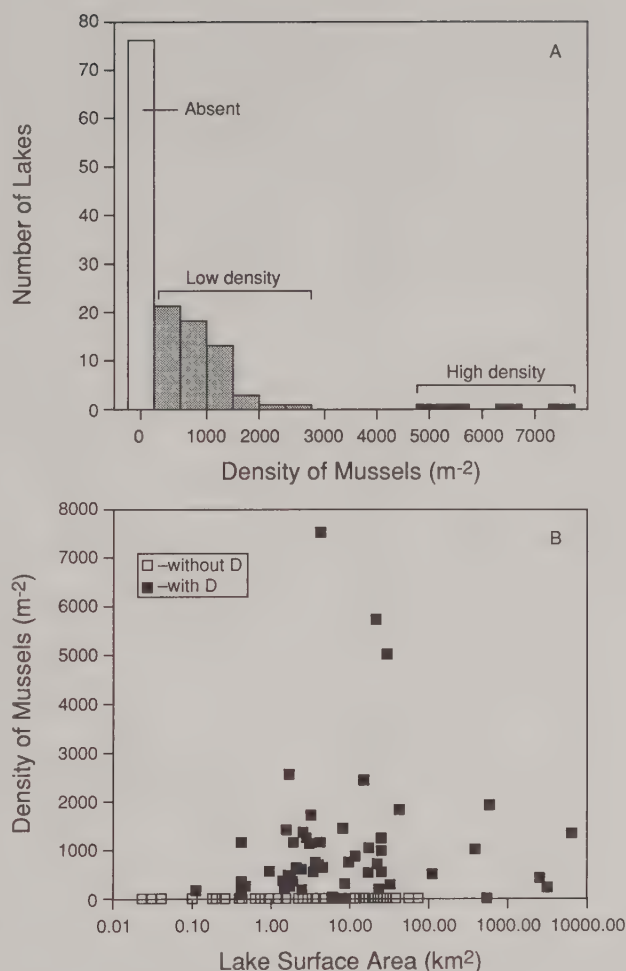


FIG. 1. (A) Density–frequency distribution of *Dreissena* from European lakes used in our data set. The open bar indicates lakes from which *Dreissena* were absent. (B) Relationship between lake surface area (log₁₀ scale) and *Dreissena* density.

were unrelated to density of *Dreissena* and were unimportant in our predictive models. Lakes without *Dreissena* had a tendency towards smaller surface area than lakes with *Dreissena* (Fig. 1B; Table 1). Other morphological and physical variables, such as lake volume, maximum depth, mean depth, temperature, Secchi transparency, and oxygen, appear unrelated to *Dreissena* density; the stepwise DFA found that lakes with *Dreissena* did not differ morphometrically from those without.

The three final models are summarized in Table 4. The only variables necessary for predicting *Dreissena* occurrence and density in the DFA models were [Ca], pH, [PO₄], and [NO₃] (Tables 2, 3; Fig. 2). Frequency distributions of these four variables did not differ significantly from normal (Kolmogorov–Smirnov test of normality, $p \geq 0.004$, $\alpha = 0.03$ after adjustment by Bonferroni's inequalities for multiple comparisons; Snedecor and Cochran 1980). The occurrence model (No. 1 in Table 4) used pH and [Ca] to distinguish lakes with *Dreissena* from those without, with 92.7% accuracy (cross-validation error rate). In our data set, no lakes with *Dreissena* had pH values below 7.3 or [Ca] below 28.3 mg·L⁻¹ (Fig. 2A, 2B).

In the DFA model that predicts density (model 2 in Table 4; Fig. 3), the variables pH and [Ca] were important for distin-

TABLE 1. Morphometric characteristics and *Dreissena* abundance of lake groups used in the DFA. Density is *Dreissena* abundance, length is longest dimension, SE is standard error, and *n* is sample size.

	Density (no·m ⁻²)	(Drainage) basin area (log(km ²))	Lake surface area (log(km ²))	Lake volume (log(10 ⁶ m ³))	Maximum depth (m)	Mean depth (m)	Epilimnion depth (m)	Length (km)	Max. width (km)
Presence-absence model									
<i>Dreissena</i> absent									
Mean	0.00	2.74	0.384	2.223	43.018	15.243	4.896	23.490	1.023
SE	0.000	0.187	0.341	0.726	14.703	5.288	0.610	6.552	0.234
Min.	0.00	2.23	-1.620	-0.854	2.900	1.500	2.900	0.230	0.150
Max.	0.00	3.25	1.852	3.872	189.90	37.000	7.000	41.000	1.950
<i>n</i>	18	5	14	7	15	7	8	7	7
<i>Dreissena</i> present									
Mean	813.53	2.583	1.21	2.26	43.40	15.79	10.687	92.48	8.772
SE	185.363	0.240	0.150	0.161	7.262	3.746	1.170	30.405	2.022
Min.	22.00	0.79	-1.10	0.23	2.00	1.00	2.00	1.50	0.75
Max.	7541.01	6.12	3.81	4.76	286.00	145.20	25.00	546.00	56.00
<i>n</i>	58	35	55	50	56	50	28	30	30
Abundance model									
<i>Dreissena</i> absent									
Mean	0.00	2.70	1.13	2.60	74.19	12.78	3.27	25.56	1.012
SE	—	0.236	0.391	0.725	22.353	5.392	—	7.328	0.184
Min.	0.00	2.23	-0.40	-0.23	3.27	1.50	3.27	1.00	0.55
Max.	0.00	3.25	1.75	3.87	132.00	32.00	3.27	41.00	1.45
<i>n</i>	5	4	5	5	5	5	1	5	5
Low abundance									
Mean	878.36	2.35	1.31	2.32	47.90	18.53	12.49	101.06	9.26
SE	103.601	0.265	0.210	0.243	12.916	6.291	1.483	47.816	1.940
Min.	2.00	1.05	-0.39	0.23	2.00	1.10	2.00	4.53	1.18
Max.	1979.29	6.12	3.81	4.76	286.00	145.20	25.00	546.00	27.000
<i>n</i>	27	24	27	26	27	26	19	14	14
High abundance									
Mean	3492.56	2.701	1.76	2.80	45.25	11.03	5.67	139.10	16.48
SE	1281.214	1.027	0.565	0.620	10.065	3.425	0.667	86.819	10.190
Min.	3.00	0.79	0.23	1.04	18.60	5.50	5.00	3.50	0.80
Max.	7541.01	4.75	3.66	4.41	73.00	22.80	7.00	430.00	56.00
<i>n</i>	6	4	6	5	6	5	3	5	5

guishing lakes without *Dreissena* from those with low density, while $\ln([\text{PO}_4])$ and $\ln([\text{NO}_3])$ were used to discriminate between lakes with low density and those with high density (Table 5). The overall success rate of this model was 76.3% (Table 6). The model correctly classified 80.0% of the lakes without *Dreissena*, 74.1% of the low-density lakes, and 83.3% of the high-density lakes.

The regression-based density models used the variables pH, $[\text{PO}_4]$, and $[\text{NO}_3]$ to predict *Dreissena* density (Table 7). pH values above the threshold of about 7.3 were positively related to *Dreissena* density (Fig. 2B). $\ln([\text{PO}_4])$ and $\ln([\text{NO}_3])$ were negatively related to *Dreissena* density (Fig. 2C, 2D) and were also strongly correlated with each other. Therefore, only $\ln([\text{PO}_4])$ (the stronger predictor) and pH were used in the final regression model (Table 7, equation D; model 3 in Table 4).

Both the occurrence and density DFA models predicted that *Dreissena* will be present in all Great Lakes basins including Green Bay, except for Lake Superior. Except for Lake Huron, all of the Great Lakes had values of the discriminant function that strongly categorized them as lakes with or without *Dreissena*. The intermediate value for Lake Huron suggested that this lake cannot be confidently classified. The density DFA model predicts that *Dreissena* will form dense populations in western Lake Erie and Lake Ontario, while all other basins (except Superior) will have low-density populations. The regression-based density model predicted densities of 1200 and

3200 mussels·m⁻² for Lake St. Clair and western Lake Erie, respectively.

Discussion

Human activity has strongly influenced zebra mussel distribution. Pleistocene deposits show that *Dreissena* was once widely distributed throughout Europe (Stańczykowska 1977) and Britain (Tate 1866). During the last glaciation, this mussel was restricted to areas around the Caspian and Aral seas, and the lower Volga river, where it was first discovered and described by Pallas (1771) (*Mytilus polymorphus*, Tate 1866). It is possible that *Dreissena* would have again extended its European range through natural colonization. However, this range expansion occurred within only 200 yr due to extensive ship traffic and connection of drainage basins by construction of shipping canals (Schlesch 1930). Most recently, the accidental introduction of *Dreissena* into the Laurentian Great Lakes (Hebert et al. 1989) will now allow this bivalve to colonize the freshwaters of North, Central, and South America.

Thus far, *Dreissena* has been very successful at colonizing North American lakes. Within 4 yr of the initial discovery in Lake St. Clair, colonies have appeared in all the Great Lakes, adjacent smaller lakes, and the Mississippi and Hudson rivers (Sea Grant 1992). Recent population densities in western Lake Erie are among the highest ever reported (3.0×10^4 mus-

TABLE 2. Physical characteristics of lake groups used in the DFA. Temperatures and oxygen were measured at maximum depth. SE is standard error and *n* is sample size.

	Max. summer bottom temp. (°C)	Max. summer surface temp. (°C)	Min. summer bottom oxygen (mg·L ⁻¹)	Secchi disc transparency (m)	Average pH
Presence-absence model					
<i>Dreissena</i> absent					
Mean	9.38	20.33	6.25	5.38	7.16
SE	2.988	0.882	2.400	1.079	0.184
Min.	4.00	19.00	2.12	1.72	5.25
Max.	17.93	22.00	10.43	11.00	8.25
<i>n</i>	4	3	3	8	18
<i>Dreissena</i> present					
Mean	10.56	20.31	5.12	3.64	8.08
SE	0.961	0.319	0.664	0.457	0.051
Min.	4.00	16.00	0.00	0.40	7.28
Max.	21.00	23.00	11.27	16.20	9.00
<i>n</i>	23	26	35	41	58
Abundance model					
<i>Dreissena</i> absent					
Mean	17.93	19.00	10.43	4.71	6.81
SE	—	—	—	1.132	0.250
Min.	17.93	19.00	10.43	1.72	6.14
Max.	17.93	19.00	10.43	7.98	7.68
<i>n</i>	1	1	1	5	5
Low abundance					
Mean	10.49	20.87	3.98	3.48	8.037
SE	1.058	0.216	0.802	0.618	0.052
Min.	4.50	19.00	0.00	0.73	7.34
Max.	16.00	22.50	11.27	16.20	8.54
<i>n</i>	15	18	23	25	27
High abundance					
Mean	7.86	20.09	6.26	4.14	8.04
SE	1.663	1.464	2.130	1.043	0.194
Min.	5.50	18.37	0.00	1.21	7.53
Max.	11.07	23.00	9.50	6.90	8.65
<i>n</i>	3	3	4	5	6

sels·m⁻², Griffiths et al. 1991); density in Lake St. Clair is up to 4.5×10^2 mussels·m⁻² (Hebert et al. 1989), while populations recently established in the other Great Lakes are at lower densities. Around Lake Erie, communities are already experiencing expensive blockages of water intakes, fouling of turbines, accumulations of shells of dead *Dreissena* on beaches, and overgrowth of fish spawning beds (Griffiths et al. 1989, 1991).

The European experience shows that *Dreissena* density can vary by several orders of magnitude among different lakes and that *Dreissena* is entirely absent from many lakes to which it has very likely been introduced. For example, several of Germany's Holstein lakes did not have *Dreissena*, while the Grosser Plöner See, in the same district, had peak densities of 1.34×10^4 mussels·m⁻² (Lundbeck 1926). Among Polish lakes that had *Dreissena*, densities varied from 23 mussels·m⁻² in Lake Beldany to 7000 mussels·m⁻² in Lake Beloslawskie.

The economic and ecological impacts of *Dreissena* would only be evident in lakes that can support this mussel and would be worse in lakes with higher population densities. Therefore, useful predictions of the potential impact of *Dreissena* rely on accurate estimates of its potential density in different water bodies.

Occurrence Model

Most attempts to develop predictive models of either *Dreissena* occurrence or density have focused on limnological variables relating to water hardness. Both high calcium levels and high pH are necessary for shell production in many species of shell-bearing molluscs. For example, the zoogeographic distribution of snails in Norwegian lakes is strongly affected by water hardness (Ökland 1983). Zoogeography of unionid clams in North American lakes is also affected by calcium levels (Green 1971, 1972). Stańczykowska (1964) found no relationship between calcium level and *Dreissena* density among the lakes she studied. Our study indicated that calcium acts as a threshold variable that affects *Dreissena* density only when it is in limiting supply. *Dreissena* density varied greatly among lakes, with levels of calcium above the threshold value of 28.3 mg·L⁻¹ (Fig. 2A). All of the lakes studied by Stańczykowska (1964) had levels of calcium well above this threshold. Stańczykowska (1965) also found that density was unrelated to Secchi disk transparency and that *Dreissena* tended to be absent from small ponds. Strayer (1991) has recently found that *Dreissena* can tolerate a very wide range of temperatures.

In laboratory experiments, Sprung (1987) found that the timing and success of fertilization, as well as development of

TABLE 3. Chemical characteristics of lake groups used in the DFA. All values are reported in $\text{mg}\cdot\text{L}^{-1}$; SE is standard error and n is sample size.

	Ca^{2+}	Mg^{2+}	Cl^-	PO_4^{3-}	Total P	HCO_3^-	NO_3^-
Presence-absence model							
<i>Dreissena</i> absent							
Mean	20.31	2.82	11.25	0.01	—	—	0.02
SE	4.299	0.950	1.053	0.002	—	—	0.003
Min.	0.98	0.75	6.52	0.00	—	—	0.01
Max.	60.00	12.50	16.00	0.01	—	—	0.03
n	18	12	12	6	0	0	6
<i>Dreissena</i> present							
Mean	48.51	13.49	21.70	0.17	0.37	139.20	0.54
SE	1.801	2.644	3.360	0.061	0.245	10.103	0.202
Min.	28.33	0.70	0.13	0.00	0.01	27.33	0.01
Max.	85.84	59.22	170.00	2.15	6.23	284.00	7.80
n	58	30	51	43	25	31	41
Abundance model							
<i>Dreissena</i> absent							
Mean	7.65	3.19	8.54	0.01	—	—	0.02
SE	5.628	2.329	0.577	0.002	—	—	0.004
Min.	0.98	0.75	7.36	0.00	—	—	0.01
Max.	30.07	12.50	10.60	0.01	—	—	0.03
n	5	5	5	5	0	0	5
Low abundance							
Mean	49.09	19.69	14.14	0.09	0.14	158.97	0.29
SE	2.533	6.125	1.231	0.013	0.016	10.944	0.102
Min.	29.27	0.70	0.13	0.00	0.04	56.08	0.01
Max.	85.83	41.14	34.82	0.22	0.22	284.00	2.14
n	27	6	25	27	19	21	27
High abundance							
Mean	42.25	10.83	13.42	0.03	0.12	87.68	0.31
SE	4.343	4.446	9.647	0.019	0.102	24.330	0.171
Min.	28.33	2.50	1.40	0.00	0.01	58.56	0.01
Max.	52.73	22.68	32.50	0.12	0.22	136.00	0.92
n	6	4	3	6	2	3	6

TABLE 4. Three final models developed to predict zebra mussel occurrence or density. Ion concentrations are in $\text{mg}\cdot\text{L}^{-1}$.

Final model	Prediction	Equation
1	Occurrence	$A = 1.246\text{pH} + 0.045[\text{Ca}] - 11.696$ Mussels present if $A > -0.638$
2	Density	$D1 = 2.773\text{pH} + 0.040[\text{Ca}] - 0.016(\ln[\text{PO}_4])$ $+ 0.383(\ln[\text{NO}_3]) - 22.658$ $D2 = -0.342\text{pH} + 0.007[\text{Ca}] + 0.789(\ln[\text{PO}_4])$ $+ 0.490(\ln[\text{NO}_3]) + 5.145$ Use the scores of D1 (which is the value of discriminant function 1) and D2 (value of discriminant function 2) with Fig. 3 to make density category predictions
3	Density	$\text{Number}\cdot\text{m}^{-2} = 2469\text{pH} - 88959e^{[\text{PO}_4]} - 8773$

embryos and young larvae, were strongly affected by levels of calcium and pH. Survivorship of *Dreissena* embryos was positively related to calcium concentration and also reached a peak at pH values around 8.4. Larval mortality was almost 100% at pH values below 7.3. We found that pH 7.3 is the lower limit for occurrence of *Dreissena*. Zebra mussels may be restricted from softwater lakes because of high larval mortality. Recently, Strayer (1991) also suggested that lakes with *Dreissena* tend to have higher levels of total ions.

Our study shows clearly that *Dreissena* is restricted from

lakes that have low levels of pH and calcium (Fig. 2A, 2B). The DFA uses only these two variables to correctly predict presence or absence of *Dreissena* with 92.7% accuracy. Other factors such as lack of suitable substrate may prevent *Dreissena* from colonizing those lakes that have pH values above 7.3 and calcium levels above $28.3 \text{ mg}\cdot\text{L}^{-1}$. For example, several lakes without *Dreissena* and with high pH and calcium are Polish and Soviet reservoirs that were formed from flooded farmland. When flooded, the soil became a flocculent ooze (Pieczynska 1976; Mordukhai-Boltovskoi 1979) that cannot provide firm

TABLE 5. Coefficients and group centroids of the models that were developed by DFA. For the abundance model, function 1 primarily contrasts lakes with *Dreissena* from those with low density, while function 2 contrasts lakes with low density from those with high density.

	Variable	Coefficient function 1	Coefficient function 2
Presence-absence model	pH	1.246	
	Ca	0.045	
	Constant	-11.695	
	Centroid of "absent"	-1.849	
	Centroid of "present"	0.574	
Abundance model	pH	2.773	-0.342
	Ca	0.040	7.752×10^{-3}
	$\ln(\text{PO}_4)$	-0.016	0.789
	$\ln(\text{NO}_3)$	0.383	0.495
	Constant	-22.658	5.145
	Centroid of "absent"	-4.987	-1.331
	Centroid of "low"	0.811	0.418
	Centroid of "high"	0.505	-0.773

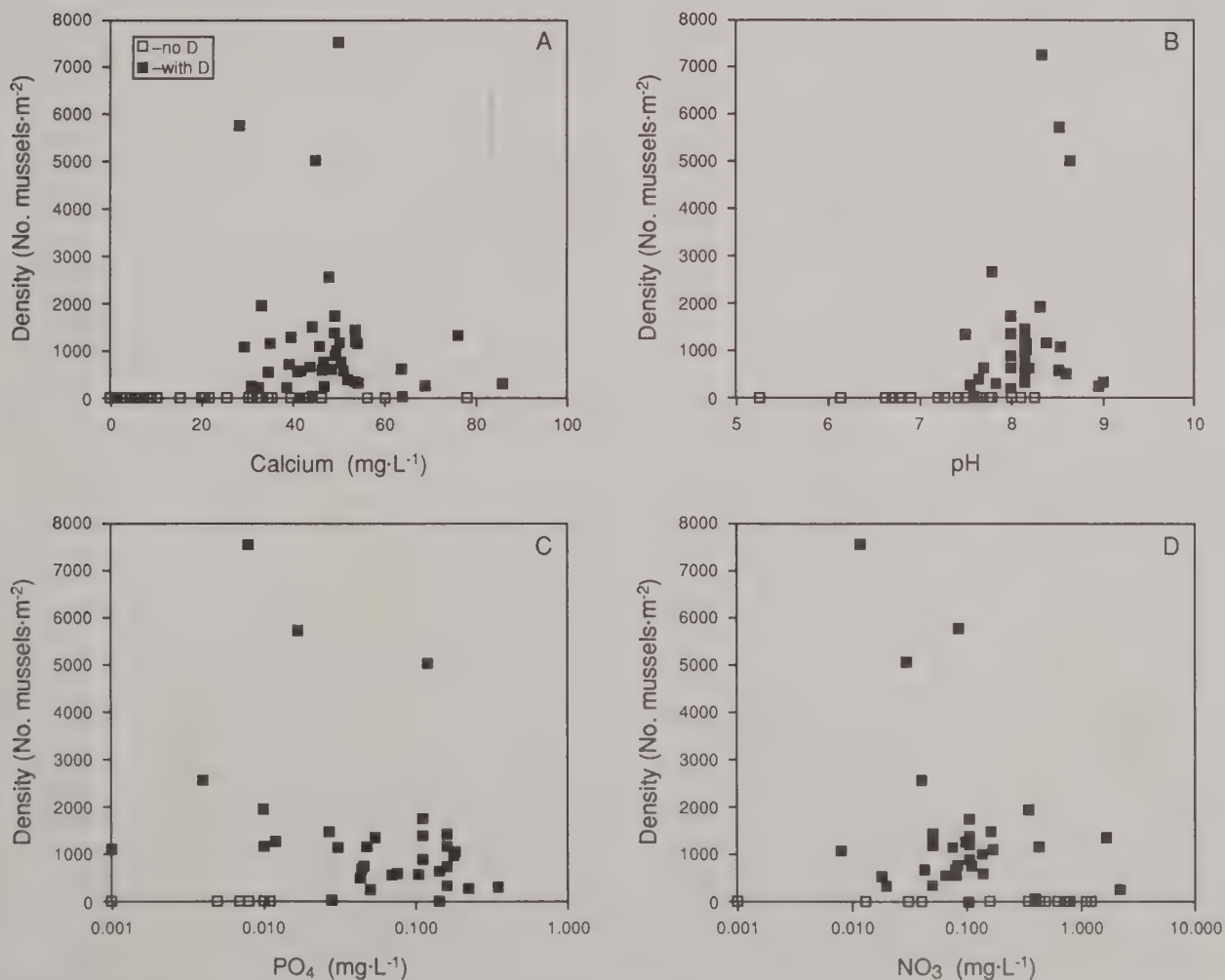


FIG. 2. Relationship between *Dreissena* density and (A) calcium concentration, (B) pH, (C) PO_4 concentration ($\log_{10}$ scale), and (D) NO_3 concentration ($\log_{10}$ scale).

TABLE 6. Cross-validation success rates (%) for the DFA model that predicts abundance. "Variables" indicates which limnological variables were important in distinguishing between two lake types. Values in parentheses are number of lakes. The overall success rate for correct classifications was 76.3%.

	Predicted			Variables
	Absent	Low	High	
Observed				
Absent	80.0 (4)	0.0 (0)	20.0 (1)	Ca, pH
Low	0.0 (0)	74.1 (20)	25.9 (7)	
High	0.0 (0)	16.7 (1)	83.3 (5)	ln(PO ₄), ln(NO ₃)

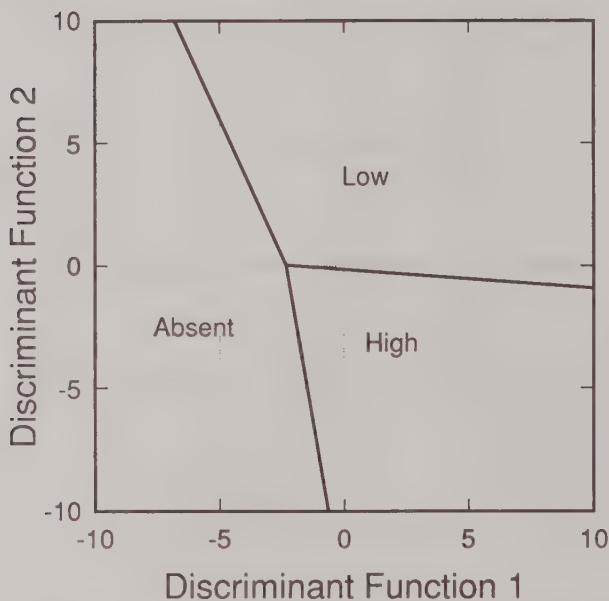


FIG. 3. Map (phase diagram) of the values of discriminant functions 1 and 2 for the model predicting density of *Dreissena* using the second model in Table 4. "High" density is over 3000 zebra mussels·m⁻².

attachment sites for *Dreissena*. In other reservoirs, *Dreissena* commonly occurs on submerged rocks and trees (Mordukhai-Boltovskoi 1979).

Density Models

Although Stańczykowska (1964) found no strong relationships between *Dreissena* density and variables such as calcium and Secchi depth, she later found some evidence that *Dreissena* tended to be rare or absent in lakes with high levels of phosphorus (Stańczykowska et al. 1983; Stańczykowska 1984). In our study, *Dreissena* density was negatively related to both ln(PO₄) and ln(NO₃), suggesting that more eutrophic lakes are less suitable environments. Eutrophic lakes may be detrimental to *Dreissena* because high densities of algae, particularly blue-green algae, may clog their ctenidia (gills for filter-feeding, Stańczykowska 1984). Lakes with high primary productivity may also have lower levels of oxygen, due to high rates of bacterial decomposition of plant material.

TABLE 7. Nonlinear multiple regression models to predict *Dreissena* density. Predictions of model D are presented in Fig. 4.

Model	r ²	p
(A) 810.619pH - 5328.334	0.534	<0.0005
(B) - 5578.540e ^{1/PO₄1} + 7540.236	0.489	<0.0005
(C) - 144.134e ^{1/NO₃1} + 1643.642	0.451	<0.0005
(D) 2469.003pH - 8894.510e ^{1/PO₄1} - 8772.579	0.594	<0.0005
(E) 2834.425pH - 7762.502e ^{1/PO₄1} + 48.849e ^{1/NO₃1} - 13025.199	0.598	<0.0005

The lack of strong correlations between *Dreissena* density and limnological variables may indicate that density is more strongly determined by ecological factors than by physiological tolerances. While presence or absence of *Dreissena* may depend on the limitations of larval development and shell growth, density may more strongly depend on factors such as predation, food limitation, substrate availability, and recruitment. Several species of fish (e.g. Plizska 1956; Biró 1974) and waterfowl (e.g. Borowiec 1983) can consume substantial proportions of *Dreissena* production, although the ability of predators to control *Dreissena* populations has not been thoroughly studied (Stańczykowska 1990). The effects of parasites (Dobrzańska 1958) are also unknown (Stańczykowska 1977). Larval recruitment may be an important population bottleneck for *Dreissena* (Walz 1978b; Lewandowski 1982) as it is for many marine invertebrates (Underwood and Denley 1984). Variability in *Dreissena* density among different lakes may be caused by differences in factors that affect larval mortality such as lake morphometry, temperature regimes, wind mixing (Lewandowski 1982), adult population density (MacIsaac et al. 1991), and substrate availability.

Interlake differences in abundance of algal food may or may not affect *Dreissena* density. This mussel can survive and grow at low food levels (Walz 1978a, 1978b; Sprung and Rose 1988). In laboratory experiments, both filtration and consumption rates follow a Type 2 functional response curve (Walz 1978a; Sprung and Rose 1988). However, consumption rate reaches a maximum at low food levels (1.6×10^4 cells·mL⁻¹, Sprung and Rose 1988; 2.0 mg carbon·L⁻¹, Walz 1978a), while filtration rate continues to increase; excess filtered algae and rejected, inedible particles are expelled in pseudofeces. Even fairly oligotrophic lakes typically have phytoplankton concentrations of 1–5 mg carbon·L⁻¹ (Wetzel 1983), well within the range to support *Dreissena*.

The uncertainty in estimation of zebra mussel density in different lakes may affect the accuracy of our models. We had only a single estimate of *Dreissena* density for approximately half of the lakes in the data set that had zebra mussels. Because *Dreissena* densities fluctuate from year to year, single estimates may not accurately represent average, long-term density. Using single density estimates for lakes with fluctuating populations would probably have affected the regression models more than the DFA models, since the latter models assign lakes to broad density categories. Dense populations tend to fluctuate the most (Stańczykowska et al. 1975; Ramcharan et al. 1992); therefore, uncertainty in density estimates was greatest in lakes that should have had the strongest statistical influence on regression analyses. Moreover, we could find data for only a few lakes with high *Dreissena* densities, which probably caused the regression model to underestimate high densities (Fig. 4).

The difference in accuracy between our DFA and regression-based models may indicate the degree to which other factors, such as pollutants, predation by ducks and fish, and availability

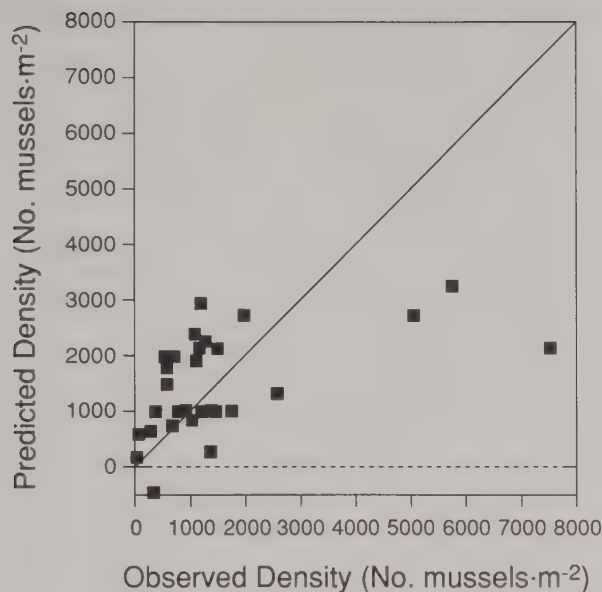


FIG. 4. Comparison of observed density of *Dreissena* and predictions of the regression-based abundance model (Table 7, equation D). The diagonal line ($y = x$) represents 100% accuracy of the model.

of firm substrate, affect the *Dreissena* density. Our occurrence and density models that were based on DFA used the variables pH, calcium, nitrate, and phosphate to obtain highly accurate predictions of *Dreissena* density levels with a low unexplained variance. The effects of factors other than the four chemical variables were apparently too weak to affect the categorization of lakes used in the DFA. For example, pollutants, shortage of firm substrate, and predation are unlikely to completely eliminate *Dreissena* from a lake. The density model that was based on multiple regression was less accurate than the DFA models and had a larger unexplained variance. Therefore, the effects of factors other than pH, calcium, nitrate, and phosphate are more evident at this finer scale.

We had also expected that substrate surface area would limit *Dreissena*, so we had searched for data on littoral zone area. Unfortunately, these data were scarce, and preliminary regression-based models that we had attempted showed no relationship between *Dreissena* density and littoral zone area. We did find many values for mean depth of our lakes and this variable should be statistically related to littoral zone area (deep lakes have small littoral zones and vice versa). We found that mean depth was not an important variable in any of our models.

Substrate quality as well as quantity may be important to *Dreissena*. Because they can only attach to firm materials, littoral zones made of sand and mud may restrict or reduce *Dreissena* populations. The growth habit of *Dreissena* in Europe indicates that availability of hard substrate is probably more important early in an invasion than later. In lakes with little hard substrate, *Dreissena* initially colonizes unionid clams, sticks and logs, and aquatic macrophytes. Veligers settle and grow on these early colonizers forming aggregations or druzes. Eventually, a *Dreissena* "mat" may form over the original soft substrates. These mats have been reported in Polish and Dutch lakes. The lakes in our data set have been colonized for so long that *Dreissena* would have had ample opportunity to form mats of hard substrate.

Our models to predict *Dreissena* occurrence and density should be directly applicable to North American lakes. The models were developed using typical temperate-zone lakes in terms of morphometry and physicochemical environments. It is unlikely that evolutionary founder effects have yet resulted in physiological differences between European and North American populations of *Dreissena* (Hebert et al. 1989). North American populations show a wide genetic diversity; they may have originated from either several separate introductions or one very large introduction (Hebert et al. 1989; May and Marsden 1992). However, it is possible that zebra mussel populations will eventually evolve genetic adaptations to local ecological conditions, as are found in the marine blue mussel, *Mytilus edulis* (Gartner-Kepkay et al. 1980; Dickie et al. 1984).

Predicting the performance of *Dreissena* populations in very large lakes such as the Great Lakes is problematic because of heterogeneity in the chemical and physical environments of these basins. Chemical heterogeneity results from both natural and man-made influences. Each Great Lake spans a range of basin geology that has important effects on water chemistry (e.g. Adams 1972; Bahnick et al. 1972). Water mixing patterns are complex. Large inputs of several different nutrients and ions often occur at river mouths, near large urban centers, and all along the shoreline through surface runoff (Neil and Jackson 1982). Therefore, within each lake, water chemistry may vary greatly among different nearshore areas that *Dreissena* typically inhabits (Kinkead and Chatterjee 1974). Predictions can be improved using data from smaller subunits of each Great Lake, rather than average values for the entire lake as we have done for the present analysis. A good example is our prediction that *Dreissena* would be absent from Lake Superior, yet populations of zebra mussels are already found in Superior Harbour. Our prediction was based on chemical data for offshore stations in Lake Superior (Adams 1972). The water in Superior Harbour, influenced by outflow of the St. Louis River, has higher levels of calcium than the open lake and therefore would be predicted as zebra mussel habitat by our models.

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Life History of Juvenile Ocean-type Chinook Salmon (*Oncorhynchus tshawytscha*) in the Situk River, Alaska

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Distribution, abundance, habitat preference, migration and residence timing, seawater tolerance, and size were determined for juvenile ocean-type (age 0) chinook salmon (*Oncorhynchus tshawytscha*) in the Situk River, Alaska. Chinook primarily occupied main-stem habitats (channel edges in spring, pools and willow edges in summer). Peak chinook densities in the upper and lower main stem were 96 and 76 fish/100 m², respectively. Chinook migrated downstream in two phases: a spring dispersal of emergent fry and a summer migration. Chinook marked in the upper river in late June and early July were recaptured 20 km downstream in the lower river in late July. Marked chinook resided in the lower river up to 34 d. Mean fork length of chinook in the lower river increased from 40 mm in May to 80 mm in early August. By late August, chinook had emigrated from the lower river at a size of approximately 80 mm. Fish this size were seawater tolerant and had the physical appearance of smolts. Ocean-type chinook in the Situk River are unique because in most Alaskan streams, chinook are stream-type (rear in freshwater at least 1 yr).

Nous avons déterminé la répartition, l'abondance, les préférences en matière d'habitat, le moment des migrations et des périodes de séjour, la tolérance à l'eau de mer ainsi que la taille du saumon quinnat juvénile de mer (*Oncorhynchus tshawytscha*) (moins de 1 an) dans la rivière Situk, en Alaska. Le quinnat fréquente surtout les habitats compris dans le bras principal de la rivière (le bord des chenaux au printemps, les fosses et les rives bordées de saules en été). Les densités maximales de saumon quinnat dans les parties supérieure et inférieure du bras principal de la rivière s'élevaient à 96 et à 76 individus/100 m² respectivement. Le quinnat a migré en aval en deux étapes : d'abord la dispersion du frai qui venait d'éclore au printemps, puis la migration estivale. Les saumons quinnats marqués dans la partie supérieure de la rivière à la fin de juin et au début de juillet ont été recapturés 20 km plus bas dans la partie inférieure de la rivière à la fin de juillet. Les saumons marqués sont restés dans cette partie de la rivière jusqu'à 34 jours. La longueur moyenne à la fourche des saumons quinnats dans la partie inférieure de la rivière est passée de 40 mm, en mai, à 80 mm au début d'août. À la fin d'août, les quinnats avaient émigré de la partie inférieure de la rivière, leur taille étant d'environ 80 mm. Les spécimens de cette taille toléraient l'eau de mer et avaient l'apparence physique de smolts. Les saumons quinnats de mer de la rivière Situk sont uniques parce que dans la plupart des cours d'eau de l'Alaska, les saumons quinnats sont des saumons de rivière (élevés en eau douce pendant au moins 1 an).

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Juvenile chinook salmon (*Oncorhynchus tshawytscha*) in Alaska typically exhibit a "stream-type" life history (Healey 1983); fish rear in freshwater for at least 1 yr before migrating to sea as smolts (Kissner 1986; Van Alen et al. 1987). In the Situk River in northern Southeast Alaska, however, limited studies (Kissner 1986) have indicated that most chinook may be "ocean-type" (migrate to sea at age 0 without spending one or more winters rearing in freshwater (Healey 1983)) and not stream-type as previously thought. Occurrence of ocean-type chinook in the Situk River would counter Healey (1983) and Taylor (1990) who reported that only stream-type chinook occur north of the Nass River, British Columbia.

Most life history information on ocean-type chinook has been collected from the Pacific coast of the United States and British Columbia (Columbia, Sixes, Nanaimo, Sacramento, and Fraser rivers) (Rich 1920; Reimers 1971; Healey 1980; Kjelson et al. 1982; Levy and Northcote 1982). A comprehensive summary of ocean-type chinook life history is provided in Healey (1991). Variations exist in the life history of ocean-type chinook. Some chinook migrate to sea shortly after emergence from the gravel

and rear in estuarine habitats whereas others rear in freshwater 2–3 mo before migrating to sea (Carl and Healey 1984). Objectives of our study were to document the existence and describe the life history of ocean-type chinook in the Situk River by examining the distribution, abundance, habitat preference, migration and residence timing, seawater tolerance, and size of juveniles before seaward migration.

Study Area

The Situk River, 18 km southeast of Yakutat, Alaska (Fig. 1), is a clear, groundwater-fed, medium-sized coastal stream. The main stem is 35 km long from the outlet of Situk Lake (315 ha) to the ocean; peak discharge can exceed 60 m³/s (Lamke et al. 1991). The "lower" river, defined as the 3.5 km of the main stem before it enters the estuary basin (Fig. 1), is influenced by tides daily. At high tide, the lower river deepens, water velocity subsides, and salinity increases but remains low (mean bottom salinity <5.0‰) (Heifetz et al. 1989). The remainder of the main stem, unaffected by tides,

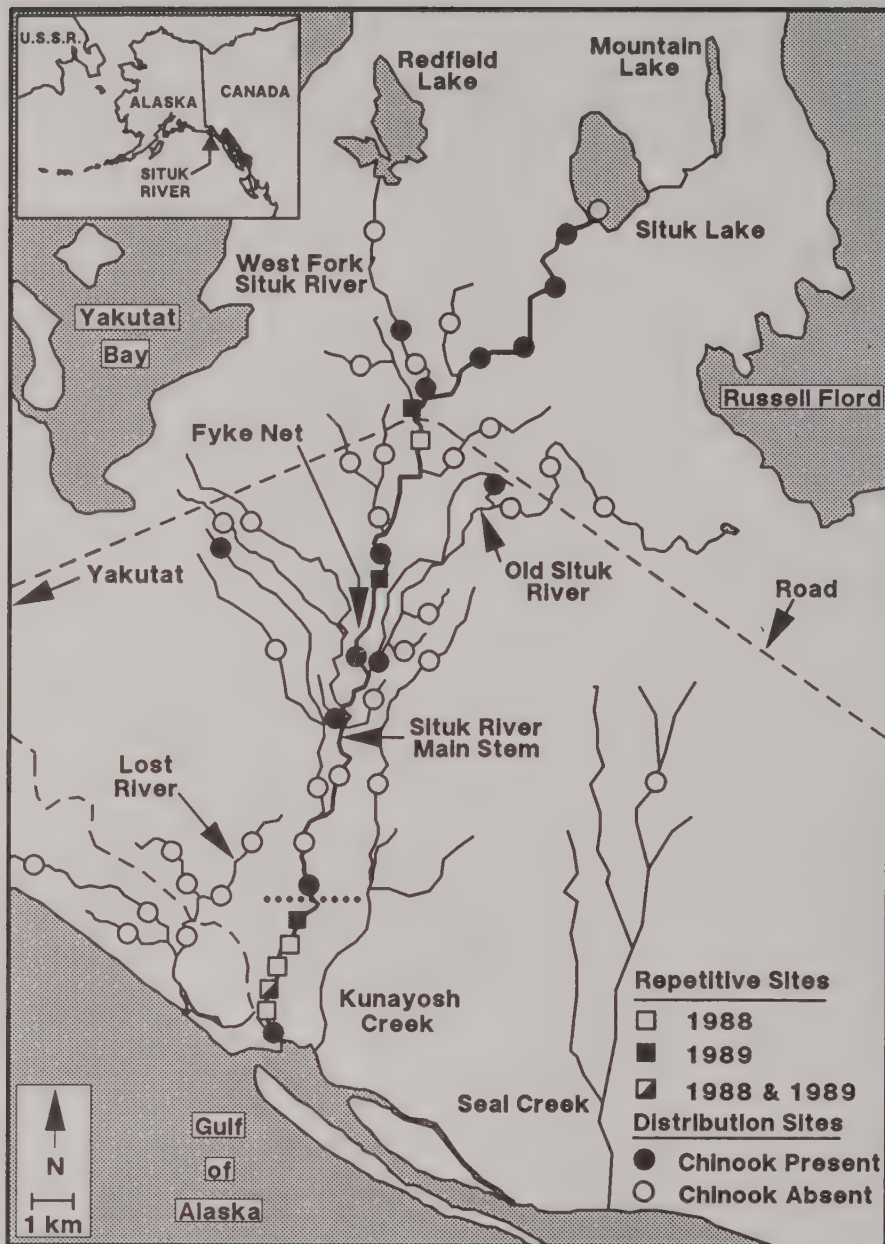


FIG. 1. Sites sampled for juvenile chinook salmon in the Situk River and neighboring watersheds, 1988–89. Sites were either repetitively sampled in a given year or were sampled only once (distribution sites). Chinook were present in all repetitive sites. The dotted line across the lower Situk main stem represents the upper limit of tidal influence and the boundary between the upper and lower sections of the river.

was considered the “upper” river. The Situk River has two major tributaries: Old Situk and West Fork Situk rivers. Old Situk River, 20 km long, originates at a small pond and enters the main stem 17 km upstream of the mouth; West Fork Situk River, 10 km long, flows out of Redfield Lake (200 ha) and enters the main stem 21 km upstream of the mouth. Neighboring watersheds include Kunayosh Creek (1 km east of Situk River), Seal Creek (8 km east), and Lost River (2 km west) (Fig. 1). Peak discharge may reach 10–15 m³/s in the Lost

River, the largest of these three watersheds.

The Situk River contains stocks of five Pacific salmon (*Oncorhynchus* spp.) and steelhead trout (*O. mykiss*), cutthroat trout (*O. clarki*), and Dolly Varden (*Salvelinus malma*). It is a medium-sized chinook production system: average annual total run size was 2450 fish from 1976 to 1990 (S. McPherson, Alaska Department of Fish and Game, P.O. Box 240020, Douglas, AK 99824-0020, USA, pers. comm.). Most chinook spawn in the upper main stem below Situk Lake and above the

confluence with West Fork Situk River (J. M. Lorenz, NMFS Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801-8626, USA, pers. comm.).

Methods

This study reports data collected in 1988 and 1989 as part of an investigation on the potential impacts of flooding from Russell Fiord (from the advance of Hubbard Glacier) on Situk River fishery resources (Clark and Paustian 1989). Juvenile chinook were sampled at 55 sites in the Situk River and neighboring watersheds (Fig. 1). Sites were either repetitive (8) or distribution (47); repetitive sites were sampled several times within a given year to determine seasonal changes in chinook size and abundance whereas distribution sites were usually sampled only once (March–October) to document the presence or absence of chinook. In 1988, repetitive sites included four in the lower river and one in the upper river; sites were sampled about every 3 wk from 13 April to 1 September (sampling at the upper river site did not begin until mid-May). In 1989, repetitive sites included two in the lower river and two in the upper river; sites were sampled about every 2 wk from 10 May to 22 September. Repetitive sites were different in 1988 and 1989, except for one site in the lower river. Repetitive sites sampled in spring and summer 1989 were also examined in late November 1989. Similar habitats were sampled and comparable methods (e.g. seines, minnow traps) were used at both repetitive and distribution sites.

In 1988, at repetitive sites, relative abundance was determined from total catch of chinook by habitat type. At least one channel edge (margin of main channel with little or no overhanging vegetation), debris pool (pool with large woody debris (LWD); pieces ≥ 10 cm in diameter and 1 m long), and main-stem channel pool (deep area of main-river flow, usually void of LWD) were usually sampled at each of the five sites. Chinook in channel edges were captured with a pole seine (5.4 m long, 1.5 m deep, and 6-mm mesh) pulled parallel to shore for 20 m (area seined 74 m²). Minnow traps baited with salmon roe were placed in debris pools. Five to 10 traps were spaced 3–5 m apart throughout the pool and set for 30 min. Chinook in main-stem channel pools were captured with a beach seine (28 m long and 3 m deep with 13-mm stretch mesh on the wings and a central bag of 6-mm stretch mesh). Researchers on foot set the seine parallel to and 3–4 m from shore and retrieved it from shore. Only one pass with a seine was made at channel edges and main-stem channel pools.

We sampled more intensively at repetitive sites in 1989 than in 1988 primarily to (1) determine population sizes, (2) determine seasonal changes in abundance in the upper and lower river, and (3) document the emigration of chinook from the river. During each sampling period in 1989, three channel edges, one debris pool, and one willow edge (margin of main channel with dense overhanging willow (*Salix* sp.) and other vegetation) were usually sampled at each of the four repetitive sites. Channel edges were seined similarly to 1988 sites, except that at least three passes with a seine were made per channel edge in 1989. Minnow traps were placed in debris pools; 10–15 traps were set approximately 3–5 m apart throughout the pool. Traps were set for 40 min, retrieved, emptied of fish, rebaited, and set again in the same location for 40 min. Traps were usually set a total of four times (range three to six times). Willow edges were sampled similarly to debris pools; a 21-m section of willow edge was selected and seven minnow traps (spaced 3 m apart) were set for 40 min, retrieved, emptied of

fish, rebaited, and set again. For debris pools and willow edges, the first trap was set 3 m upstream from the lower boundary of each habitat type.

In 1989, at repetitive sites, population sizes of juvenile chinook were estimated in channel edges, debris pools, and willow edges by the removal method (Zippen 1958). Probability of capture was assumed constant, and immigration and emigration were assumed negligible during sampling. Because sampling occurred during the day, the assumption of minimal fish immigration or emigration during sampling was reasonable; after emergence, chinook typically disperse downstream mainly at night (Healey 1991). In addition, we placed the first minnow trap 3 m upstream from the lower boundary of willow edges and debris pools to limit attracting fish from downstream areas. Total catch of juvenile chinook was used in the analysis instead of the estimated N if the estimated probability of capture (q) was ≤ 0.20 . Fish density for each habitat type was calculated by dividing the population estimate by the area seined or trapped.

In 1989, the amount of available habitat was measured at each habitat type at each repetitive site during low flow and in the lower river at low tide. Total area of debris pools and willow edges was estimated by multiplying mean width (measured at 3-m transects) by total length of habitat. For debris pools, area was based on the length and width of the pool containing LWD; for willow edges, area was based on the distance willow vegetation protruded over the river. Water depth was measured at one quarter, one half, and three quarters of the way across each 3-m transect of each habitat type. Water velocity was measured at one quarter, one half, and three quarters of the way across the lower, middle, and upper transects of each habitat type. Total pieces of LWD were counted in each habitat type. Water temperature was measured every 2 h with continuous recording ENDECO¹ thermographs in 1989–90; one thermograph was located in the lower river and one in the upper river at the outlet of Situk Lake.

To assess fish residence time and movement between the upper and lower river in 1989, some juvenile chinook were marked with a dye (Alcian Blue) injected into the caudal fin with a pressurized jet from a Panjet medical instrument. Fish from upper-river repetitive sites were marked in the upper lobe of the caudal fin (UC = upper caudal) whereas fish from lower-river repetitive sites were marked in the lower lobe (LC = lower caudal). All marked fish were released at their capture site. Downstream movement of juvenile chinook was also monitored by periodically placing a fyke net (1.2 × 1.2 m, 6-mm mesh) in the main stem of the Situk River from April to late June 1989. The fyke net was set overnight (12 h) in the upper river (17 km upstream from the mouth, Fig. 1) approximately every fifth night.

Salinity tolerance bioassays (96 h) were used to test the osmocompetence of juvenile chinook. From May to July 1988, chinook were collected in the lower and upper river and placed in 60-L plastic containers filled with aerated water at 0, 26, 28, and 30‰ salinity at ambient temperature. Ocean water was mixed with freshwater to obtain desired salinity. To avoid overcrowding, fewer than 20 fish were placed in each container. Dead fish were removed every 12 h.

A random sample of juvenile chinook captured during each sampling period was measured for fork length (FL) to the nearest millimetre. Scale samples for ageing were taken from a

¹Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

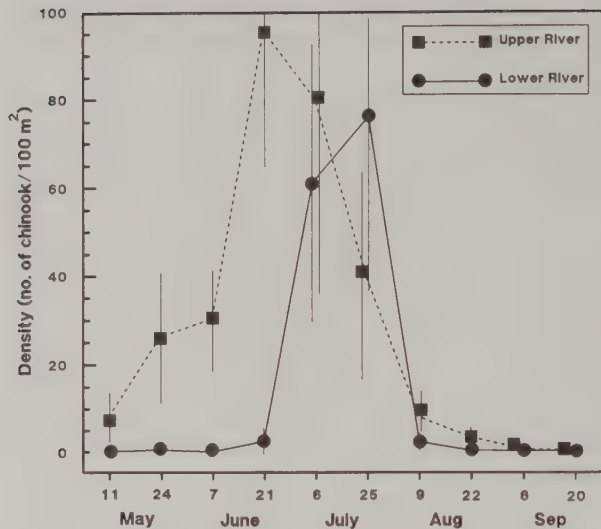


FIG. 2. Mean density ($\pm$ SE) of juvenile chinook salmon by sampling period in repetitive sites in the upper and lower Situk River, Alaska, 1989. For each data point, $n = 10$ (2 sites $\times$ 3 channel edges, 1 willow edge, and 1 debris pool per site).

range of sizes in the catch. Age-0 fish were young-of-the-year and age-1 fish had one winter annulus.

In mid-July 1989, over 10 000 juvenile chinook were captured in the lower river, coded-wire tagged, adipose (AD) clipped, and released. Mean growth rate was determined from the recapture of AD-clipped fish in the lower river in early August 1989.

Results

Distribution

Juvenile chinook were captured in only 22 of 55 sites sampled in the Situk River and neighboring watersheds (Fig. 1). Most sites with chinook were restricted to or near larger streams; 16 sites with chinook were on the main-stem Situk River (average width 27 m), and three of the six remaining sites with chinook were on tributaries just upstream (approximately 200 m) of the main stem (Fig. 1). Chinook were not captured in two main-stem distribution sites (Fig. 1), probably because these sites were sampled after late July and most chinook had already migrated to sea. Other distribution sites were accessible and contained numerous juvenile salmonids; chinook were probably absent from most, however, because of small stream size (average width 8 m).

Seasonal Abundance in the Upper and Lower River

In 1988, juvenile chinook were present at the upper river site from mid-May to 1 September. Total catch of chinook was greatest in mid-May, when nearly 50 fish were captured. In 1989, chinook were present at the upper river sites from mid-May to late September; peak density was 96 fish/100 m² in late June (Fig. 2).

In 1988, juvenile chinook were present at the lower river sites from mid-April to early August. Total catch of chinook was greatest in late June, when nearly 300 fish were captured. In 1989, chinook were present at the lower river sites from late May to late September (Fig. 2); a few newly emerged chinook

were captured in mid-March during distribution sampling. In 1989, density of chinook peaked in the lower river sites in late July (76 fish/100 m²; Fig. 2).

Chinook abundance declined by late summer: catches declined to zero fish in the lower river sites and one in the upper river site on 1 September 1988. Densities declined to only 0.03 fish/100 m² in the lower river sites and 0.55 fish/100 m² in the upper river sites on 20 September 1989 (Fig. 2). In November 1989, no chinook were captured at either the upper or lower river sites.

Habitat Utilization

Habitat characteristics (water depth, velocity, cover, and temperature) differed among habitat types (Table 1). Average depth was greatest in pools (1.2 m) and least in channel edges (0.3 m). Average water velocity was greatest in willow edges (15 cm/s) and least in pools (10 cm/s). Instream cover was abundant in pools (LWD) and willow edges (overhanging vegetation and roots in the water) and scarce in channel edges. In summer, water was consistently warmer in the upper than in the lower river (Fig. 3), probably because of the warming influence of Situk Lake. In winter, Situk Lake also moderated upper-river temperatures, which never reached 0°C. In both the upper and lower river, mean daily water temperature was consistently above 9.0°C from mid-June to September, peaking at about 20.0°C in the upper river in July (Fig. 3).

Chinook densities did not differ significantly ($P > 0.05$, Friedman's test) between lower-river habitat types, but did differ significantly ($P < 0.05$) between upper-river types. Few chinook were in the lower river in May, June, August, and September 1989; however, chinook were abundant in July, and mean densities ranged from 43 fish/100 m² in debris pools to 141 fish/100 m² in willow edges (Fig. 4). Recently emerged chinook (mean FL 43 mm), captured primarily along channel edges in May 1989, indicated that populations had not yet reached an equilibrium among habitat types in the upper river (Fig. 4). Beginning in June, however, as chinook grew (mean FL 56 mm) in the upper river, most occupied pool or willow-edge habitats, and few were found in channel edges (Fig. 4). In the upper river, the highest chinook density observed was in debris pools in July (mean 164 fish/100 m²).

Migration and Residence Timing

After emergence, chinook either dispersed downstream or remained in the upper river until July. Juveniles (mean FL 43 mm) dispersing downstream were captured by fyke net from April through June, with peak catches in May (Fig. 5); however, most fish remained in the upper river. In July, a major downstream migration of chinook to the lower river occurred: density in the lower river increased sharply from 3 to 76 fish/100 m² (21 June to 25 July 1989) whereas density in the upper river decreased from 96 to 40 fish/100 m² (Fig. 2). Further evidence of the downstream migration was the recapture of marked fish: from 19 June through 7 July 1989, 882 chinook were Panjet marked (UC) in the upper river and 37 were recaptured. Most (30) were recaptured approximately 20 km downstream in the lower river during 16–20 July (Table 2); the rest (seven) were recovered in the upper river.

Chinook were present in the lower river in substantial numbers for about 48 d (21 June to 9 August 1989; Fig. 2). Recovery of Panjet-marked fish indicated that some chinook resided in the lower river for at least 8 d and possibly as long as 34 d; of 229 chinook marked (LC) in the lower river on 22 June and

TABLE 1. Summer habitat characteristics of repetitive study sites in the lower and upper Situk River, Alaska, 1989 (P = pool with large woody debris; WE = willow edge; CE = channel edge). Values for channel edges represent the mean of three channel edges.

	Lower river						Upper river					
	Site 1			Site 2			Site 1			Site 2		
	P	WE	CE	P	WE	CE	P	WE	CE	P	WE	CE
Habitat area (m ²)	300.0	79.8	74.0	213.0	73.5	74.0	194.7	86.1	74.0	229.5	88.2	74.0
Mean depth (m)	1.2	0.8	0.4	1.5	1.0	0.3	1.1	1.2	0.3	0.8	0.6	0.3
Max. depth (m)	2.6	1.1	0.6	2.7	1.8	0.4	1.8	1.5	0.5	2.5	0.9	0.5
Mean width (m)	7.5	3.8	2.7	7.1	3.5	2.7	5.9	4.1	2.7	5.1	4.2	2.7
Water velocity (cm/s)	4	13	7	7	7	7	4	12	17	23	26	13
Pieces of LWD	8	0	0	25	0	0	9	0	0	14	0	0

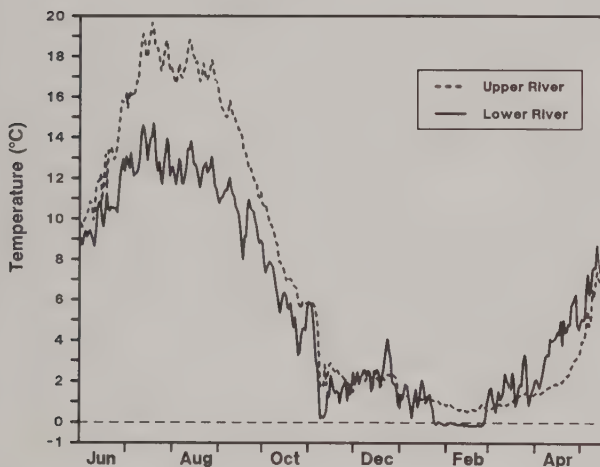


FIG. 3. Water temperature of the upper and lower Situk River, Alaska, June 1989 to May 1990.

8 July 1989, 64 were recaptured in the lower river during 16–20 July, and four were recaptured during 25–26 July (Table 2). Chinook did not migrate from the lower river to other areas in the Situk River watershed; sampling of several distribution sites (including Situk Lake) and repetitive sites after mid-August captured few chinook. Most chinook captured in the lower river in summer appeared to be smolts (silvered body and blackened fin tips).

Seawater Tolerance

Chinook from the lower river tolerated seawater earlier in the year than chinook from the upper river. Survival in 26–30‰ seawater was 91% in mid-May and 100% in early June for chinook from the lower river versus 31 and 62%, respectively, for chinook from the upper river. By mid-July, however, survival in 26–30‰ seawater was 100% for chinook from both the upper and lower river. Survival of fish of similar length from the upper and lower river differed significantly (chi-square, $P < 0.05$). In early June, survival in 26–30‰ seawater for 40–49 mm FL chinook from the lower river was 100% compared with only 64% for fish from the upper river (Table 3). Survival of ≥ 50 mm FL chinook did not differ significantly ($P > 0.05$) between the upper (90%) and lower (100%) river; in mid-July, most chinook in the river were >60 mm FL, and survival was 100%.

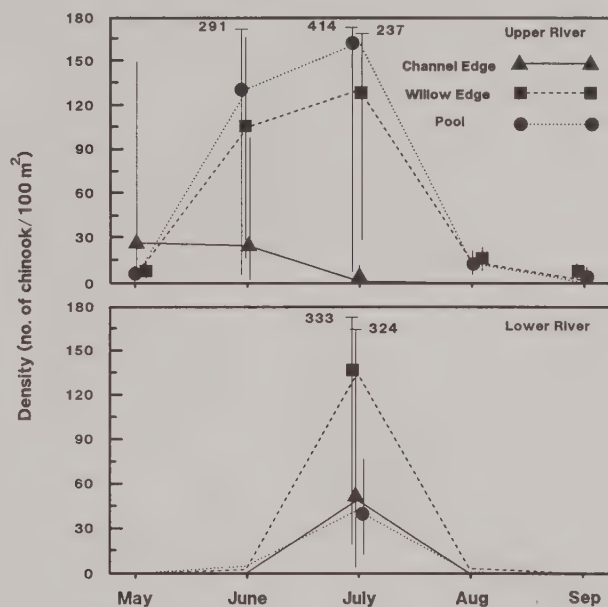


FIG. 4. Mean density ($\pm$ range) of juvenile chinook salmon by month and habitat type in repetitive sites in the upper and lower Situk River, Alaska, 1989. Channel edges, $n = 12$; willow edges, $n = 4$; pools with large woody debris, $n = 4$ (2 sampling periods $\times$ 2 sites $\times$ 3 channel edges or 1 willow edge or 1 pool per site).

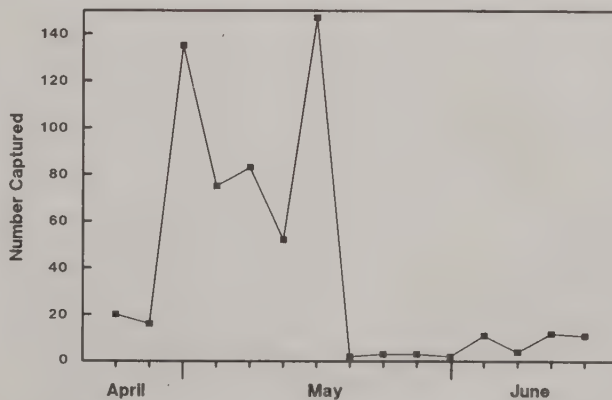


FIG. 5. Number of juvenile chinook salmon captured by fyke net in the upper Situk River, Alaska, 1989.

TABLE 2. Release and recovery of marked (Panjet and adipose clip) juvenile chinook salmon in the lower and upper Situk River, Alaska, 1989 (LC = lower caudal Panjet mark; UC = upper caudal Panjet mark; AD = adipose clip).

Date	River location	Total marks released			Marks recaptured		
		LC	UC	AD	LC	UC	AD
19–20 June	Upper		720				
22 June	Lower	40					
7 July	Upper		162			6	
8 July	Lower	189			1		
16–20 July	Lower			10 191 ^a	64	30	
25–26 July	Lower				4		155
27 July	Upper					1	
31 July	Lower						13
1 August	Lower						103
Total		229	882	10 191	69	37	271

^aCoded-wire tagged.

TABLE 3. Percent survival of similar size groups of juvenile chinook salmon from the upper and lower Situk River, Alaska, June–July 1988, after 96 h in 26–30‰ seawater. Significance based on chi-square test.

Fork length (mm)	Upper river		Lower river		P
	%	Sample size	%	Sample size	
35–39 ^a	0	5	0	0	
40–49 ^a	64	39	100	12	<0.05
50–59 ^b	90	10	100	24	NS
≥60 ^c	100	15	100	36	NS

^aFish from June sampling.

^bPredominately fish from June sampling.

^cPredominately fish from July sampling.

TABLE 4. Percent age composition of juvenile chinook salmon measured in the Situk River, Alaska, 1988–89.

Year	Period	Number aged	Age 0 (%)	Age 1 (%)
1988	Apr.–Sept.	136	97.8	2.2
1989	May–Sept.	114	98.2	1.8

Age and Size

Of the 250 chinook aged in 1988 and 1989, 98% were age 0 and 2% (5) were age 1 (Table 4). Two age-1 chinook were captured in June and three in July.

Chinook were larger in the lower river than in the upper river (Fig. 6). Most chinook reared in the upper river to about 60 mm FL before migrating to the lower river. For example, some larger chinook in the upper river in May 1989 apparently migrated to the lower river in June (range 56–76 mm FL; Fig. 6). Most chinook captured in the lower river were ≥60 mm FL (99% in 1989, 77% in 1988). Chinook reared in the lower river until they were 70–80 mm FL.

Mean size of chinook doubled in the lower river from nearly 40 mm in May to about 80 mm in early August; mean size of chinook in the upper river was <70 mm in early August (Fig. 7). Within most sampling periods, chinook in the lower river were 5–17 mm longer than chinook in the upper river. Mean size of chinook, just before abundance declined in the lower river, was 70 mm in late June 1988 and ~80 mm in late July 1989.

In late July 1989, growth rate of chinook in the lower river was ~0.57 mm/d. Based on the recapture of AD-clipped fish,

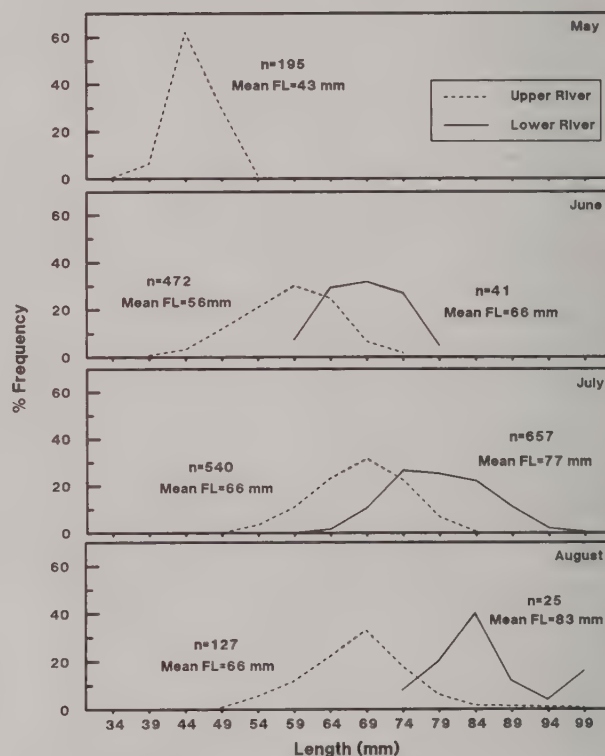


FIG. 6. Length frequency distributions of juvenile chinook salmon by month in the upper and lower Situk River, Alaska, 1989. Length frequencies are not shown where <15 chinook were measured. The x-axis represents the upper limit of 5-mm intervals.

mean FL of chinook in the lower river increased from 80 mm ($n = 423$) during 16–20 July (18 July, median release date) to 88 mm ($n = 103$) on 1 August.

Discussion

Because of the apparent presence of a freshwater annulus on adult scales, which can be difficult to identify (Koo and Isarankura 1967), most (>97%) chinook in the Situk River have been classified by fishery workers as stream-type fish (McBride

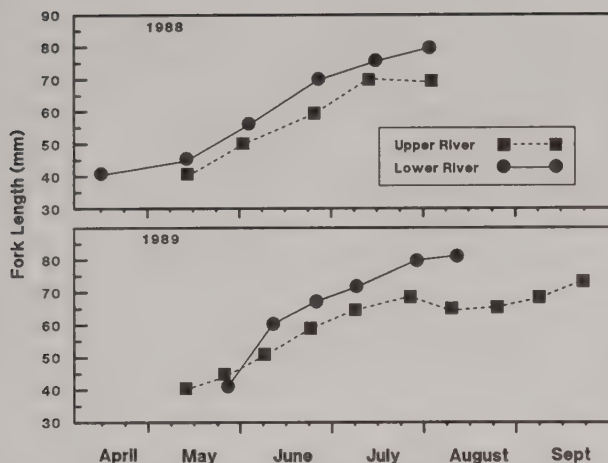


FIG. 7. Mean fork length of juvenile chinook salmon in the upper and lower Situk River, Alaska, 1988 and 1989. Each data point represents a minimum of four (range 4–381) fish measured.

1986; Riffe et al. 1987). Based on our study of juveniles in the river, we believe that most adult Situk chinook have been misidentified as stream-type. It could be argued that the disparity in freshwater age could result from only age-1 smolts (2% of the population) surviving to adulthood and poor survival of age-0 smolts (98% of the population). Recent studies, however, indicate that the total chinook smolt yield from the Situk River is ~67 000 fish (J. F. Thedinga, pers. obs.), and the approximate 2500 annual adult run could not possibly be produced by 2% of the smolt yield, even with 100% survival. The age-0 chinook we captured in the lower Situk River had the morphological appearance of smolts, could tolerate seawater, and eventually disappeared from the river; presumably they migrated to sea. Most chinook apparently do not overwinter within the Situk River watershed because few age-1 fish were present in 1988 or 1989. Kissner (1986) suggested a similar seaward migration of age-0 chinook from the Situk River in 1983 and 1984 based on juvenile sampling.

Chinook primarily occupied main-stem habitats until they apparently emigrated from the Situk River, similar to fall chinook in Sixes River, Oregon, which occupy main-stem habitats until early summer, when they migrate to the estuary (Reimers 1971; Stein et al. 1972). In spring in the upper Situk River, recently emerged chinook were often present along channel edges. By June, as fish increased in size, they moved into deeper, faster water with more cover (willow edges and debris pools). Lister and Genoe (1970) and Stein et al. (1972) also observed the shift in habitat use of juvenile chinook salmon from stream margins in spring to midstream or areas of faster water in summer. Chinook in the Situk River occupied areas with water velocity (range 4–26 cm/s) and depth (range 0.3–1.5 m) similar to areas used by chinook in other studies (Everest and Chapman 1972; Reiser and Bjornn 1979; Hillman et al. 1987).

Peak densities of chinook in the upper (96 fish/100 m²) and lower (76 fish/100 m²) Situk River were similar to densities in other studies. Murray and Rosenau (1989) reported maximum chinook densities of 6–68 fish/100 m² from May to June in tributaries of the Fraser River, British Columbia. Chinook densities in summer of 10–75 fish/100 m² were reported in some Idaho rivers (Everest and Chapman 1972; Hillman et al. 1987). In the Stikine River, Alaska, chinook densities of 2–95

fish/100 m² from May to October were reported by J. Edgington and J. Lynch (Alaska Department of Fish and Game, P.O. Box 667, Petersburg, AK 99833, USA, unpubl. data). In the glacial Taku River, Alaska, however, chinook densities (0–8 fish/100 m²; Murphy et al. 1989) were much lower than in the Situk River.

Chinook in the Situk River migrated downstream in two phases: a dispersal in spring after emergence followed by a midsummer migration. The fyke-net site was in mid-upper river, and catches probably were emergent fry redistributing to suitable rearing areas. Most chinook did not enter the lower river, however, until July, which suggests that fish remained in the upper river or migrated slowly downstream. Rearing migrations where chinook move slowly downstream throughout summer have been reported by Ewing and Birks (1982) and Beauchamp et al. (1983). Once started downstream, chinook either continue migrating directly to the estuary or stop and reside in the stream for a few weeks to a year or more (Healey 1991). The rapid increase in chinook abundance during July in the lower river with a concurrent decrease in the upper river, and the recapture of marked (UC) fish in the lower river, document a major downstream migration. After reaching the lower river, some marked (LC) chinook remained there at least 8 d to nearly a month. Residence of 1–4 wk in the lower river offers the benefits of additional food similar to estuarine conditions (Levy and Northcote 1982) and a period of seawater acclimation.

Most age-0 chinook disappeared from the Situk River by September and apparently emigrated to sea. Just before seaward migration (late June to late July), chinook mean size was 70–80 mm FL; for fish ≥60 mm FL from both the upper and lower river, survival in seawater was 100%. Weisbart (1967) reported that 70 mm FL is the approximate size at which juvenile chinook can tolerate full-strength seawater. Thus, age-0 chinook in the Situk River were of sufficient size to tolerate seawater and most likely migrated seaward. Similarly, on the Pacific coast of the United States and British Columbia, ocean-type chinook migrate to sea when about 70–80 mm FL (Healey 1980; Healey and Groot 1987).

Migration of age-0 chinook from the Situk River was slightly later (July–August) than in more southerly British Columbia streams (June–July; Healey and Groot 1987) and may vary annually depending on the severity of winter and spring. In years with a cold winter and late spring, time of emergence and growth may be retarded and time of emigration delayed; this probably accounts for the later start of emigration observed in 1989 (late July) than in 1988 (late June): average monthly air temperature from January through March 1989 was 2.6–6.5°C cooler than during the same period in 1988 (NOAA 1988, 1989). Kissner and Hubartt (1987) also reported a later out-migration of age-0 chinook in the Situk River in 1985 (August) than in 1984 (July) and attributed the later migration in 1985 to an extremely cold and late spring.

Growth of chinook in the Situk River was similar to that reported in some estuaries. Chinook rearing in the lower Situk River increased from 40 to 80 mm FL from May to early August. In some British Columbia estuaries, chinook fry increased from 40 to 70 mm FL from March to June (Healey 1980; Levy and Northcote 1982). Changes in average length of fish (over time) from the general population probably underestimate the true growth rate because of fish emigration and immigration in study areas (Healey 1991). Actual short-term (late July) growth rate, however, of marked chinook in the lower Situk River (0.57 mm/d) was similar to rates reported for

chinook fry in the Campbell River estuary, British Columbia (0.46–0.70 mm/d; Levings et al. 1986), in Coos Bay, Oregon (0.29–0.54 mm/d; Fisher and Pearcy 1990), and in the Sacramento River estuary, California (0.4–1.2 mm/d; Kjelson et al. 1982), but lower than reported by Healey (1980) for fry in the Nanaimo River estuary, British Columbia (1.32 mm/d).

Chinook in the Situk River are capable of migrating to sea as age-0 smolts, possibly because of an extended growing season. Peak spawning of chinook in the Situk River occurs the first week of September (J.M. Lorenz, NMFS Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801-8626, USA, pers. comm.); therefore, based on mean daily water temperature (Fig. 3), peak emergence of fry (calculated from heating units, 900 °C-days to emergence; Russell et al. 1983) would occur the first week of April. In other streams, peak emergence of chinook is in mid- to late April or May (Big Qualicum River (Lister and Genoe 1970), Sixes River (Reimers 1971), Taku River (Kissner 1978)). Early emergence and a longer growing season probably allow age-0 chinook in the Situk River to reach the minimum size (60–70 mm) necessary to adapt to seawater as age-0 fish.

Situk River chinook appear to be unique because they have life history characteristics between typical stream- and ocean-type populations. Most juvenile chinook emigrate from the Situk River at age 0 and at a size and time similar to ocean-type populations on the northern Pacific coast. Adult freshwater entry (June–July) and spawning timing (mid-August to mid-September), however, are more similar to stream-type populations than to ocean-type (S. McPherson, Alaska Department of Fish and Game, P.O. Box 240020, Douglas, AK 99824-0020, USA, pers. comm.).

We have documented for the first time that ocean-type life history dominates a stream population of chinook north of 56°N latitude (Taylor 1990). The only other river in Alaska with an apparent emigration of substantial numbers of age-0 chinook smolts is the Deshka River in southcentral Alaska (Delaney et al. 1982). In the Situk River, peak emergence of chinook appears to be in early April. Chinook migrate downstream in two phases: (1) a spring dispersion of emergent fry to suitable rearing areas in mid-upper river and (2) a summer migration to the lower river. Juveniles rear in and acclimate to seawater in the lower river for 1–4 wk (late June and July) before entering the main estuary by early August at ~80 mm FL. By September, chinook are virtually absent from the Situk River.

Future tag recoveries of adults returning from juveniles tagged with coded-wire tags in July 1989 should provide valuable information on the ocean distribution, survival, and exploitation rate of this unique stock. Instream recoveries of adult coded-wire-tagged chinook will also substantiate our age designations.

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Relationship between Diurnal Activity Patterns, Cryptic Coloration, and Subsequent Avoidance of Predaceous Fish by Perlid Stoneflies

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We demonstrate that prey (stoneflies), which are either solidly coloured or patterned, coordinate intervals of maximum daytime activity with periods when visually foraging predators (rainbow trout, *Oncorhynchus mykiss*) are least able to detect them. In this study, rainbow trout attacked solidly coloured stonefly nymphs (*Paragnetina media*) more frequently under conditions of low illumination (simulated dawn and dusk) than high illumination (simulated midday) whereas the frequency of attacks on patterned stonefly nymphs (*Agnatina capitata*) was reversed. Consequently, in both the presence and absence of trout, the activity (i.e. frequency of crawling) of *P. media* and *A. capitata* was highest under conditions of high and low illumination, respectively. The number of attacks by trout on all nymphs was significantly less when nymphs were on dark-brown, rather than mottled, substrates. When given a choice between dark-brown and mottled substrate, both species selected dark substrate. Suggestions that the visual acuity of predators varies as a function of light intensity and the coloration, patterning, and daytime activity of prey are corroborated for the first time.

L'étude vise à démontrer que les proies (perles), qui sont de couleur uniforme ou bigarrées, coordonnent des intervalles d'activité diurne maximales avec des périodes durant lesquelles les prédateurs qui chassent visuellement (truite arc-en-ciel, *Oncorhynchus mykiss*) sont le moins en mesure de les détecter. Dans cette étude, la truite arc-en-ciel a attaqué des nymphes de perle (*Paragnetina media*) à couleur uniforme plus fréquemment dans des conditions de faible luminosité (aube et crépuscule simulés) que sous forte luminosité (milieu du jour simulé), alors que la fréquence des attaques sur les nymphes de perle (*Agnatina capitata*) bigarrées était inversée. Conséquemment, que les truites soient présentes ou absentes, l'activité (c'est-à-dire la fréquence de rampe) de *P. media* et de *A. capitata* était respectivement la plus élevée dans des conditions de luminosité élevée et faible. Le nombre d'attaques par truite sur l'ensemble des nymphes était nettement moindre lorsque les nymphes se trouvaient sur un substrat brun foncé plutôt que marbré. Lorsqu'elles avaient le choix entre un substrat brun foncé et un substrat marbré, les deux espèces ont choisi le foncé. Les allégations à l'effet que l'acuité visuelle des prédateurs varie en fonction de l'intensité lumineuse, de la coloration, de l'arrangement des couleurs et de l'activité diurne des proies sont corroborées pour la première fois.

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Only qualitative evidence supports the suggestion that visual predators are poor at discerning prey of solid coloration under conditions of high illumination (midday) and that patterned prey are difficult to resolve under conditions of low illumination (dawn and dusk) (Edmunds 1974; Lythgoe 1979). For example, animals with disruptive patterning, such as zebras, may be most conspicuous during midday and least conspicuous under twilight conditions. Conversely, animals that are solidly coloured may be more cryptic during the day and less so at dawn and dusk. If this suggestion is correct, it is reasonable to hypothesize that prey which vary in coloration and body patterning may have evolved the behavioural capacity to coordinate peaks in their daytime activity with periods when they are least conspicuous to predators (Ives and Dobson 1987; Lima and Dill 1990).

Aquatic insects are an ideal group of organisms in which to test this hypothesis, as they show a wide range in body coloration and patterning (Merritt and Cummins 1984) and are subject to significant levels of predation by visually foraging fishes (Feltmate and Williams 1991; Williams and Feltmate 1992). Although no studies have tested whether the behaviour of aquatic insects is related to diurnal variation in crypsis, a study

by Otto (1984) lends some support. He demonstrated that under low light conditions the striped head capsules of larval caddisflies, mounted on pins, were attacked less frequently by brown trout (*Salmo trutta*) than those which were solidly coloured and that under illuminated conditions the outcome was reversed. However, mounting the heads of caddisflies on pins, without bodies (consequently negating movement), rendered Otto's findings difficult to interpret. For example, if the caddisflies had been able to move, this may have stimulated attacks by trout (Edmunds 1974), thus negating any putative advantage conveyed by disruptive or solid coloration of the head capsule.

In this paper, we use rainbow trout (*Oncorhynchus mykiss*) and two congeneric predaceous stoneflies (*Paragnetina media* (Walker) and *Agnatina capitata* (Pictet)) to test morphological and behavioural defences of aquatic insects. *Paragnetina media* and *A. capitata* are morphologically similar perlid stoneflies (Hitchcock 1974) that cohabit riffle sections of streams throughout northeastern North America (Hynes 1976). Their diets are primarily composed of caddisflies, mayflies, and chironomids (Tavares-Cromas 1990), and they feed during both day (Feltmate and Williams 1989a, 1989b) and night (Soluk and Collins 1988). *Paragnetina media* is uniformly dark brown,

TABLE 1. Mean number of attacks (per 30 min) by rainbow trout on *A. capitata* and *P. media*, under lighting conditions of low (8.0×10^{13} quanta $\cdot$ s $^{-1}$ ·cm $^{-2}$) and high (3.66×10^{15} quanta $\cdot$ s $^{-1}$ ·cm $^{-2}$) illumination, when nymphs occurred on substrates that were either dark brown, medium mottled, or light mottled (error bars = ± 1 SE).

Species	Substrate colour	Illumination	
		Low	High
<i>Agnetina capitata</i>	Dark	1.1 $\pm$ 0.8	7.2 $\pm$ 1.4
	Medium mottled	6.9 $\pm$ 3.3	14.8 $\pm$ 4.1
	Light mottled	7.1 $\pm$ 4.1	15.3 $\pm$ 3.5
<i>Paragnetina media</i>	Dark	8.1 $\pm$ 2.1	2.1 $\pm$ 1.8
	Medium mottled	14.6 $\pm$ 3.1	6.9 $\pm$ 1.1
	Light mottled	14.4 $\pm$ 2.8	8.3 $\pm$ 1.9

while *A. capitata* has alternating light and dark patches on the head, thorax, and abdomen. Previous studies have demonstrated that trout are prominent visual predators (Ware 1972; Williams 1981; Fausch 1984; Bannon and Ringler 1986; Adams et al. 1988; Moore and Gregory 1988) which can reduce density, size, and condition (weight/head width) of immature percid stoneflies and condition and fecundity of adults (Feltmate and Williams 1989a, 1991). Hence, if an antipredator response which combines morphology and behaviour does exist in aquatic insects, it is reasonable to postulate that this defence should exist in stoneflies, which are clearly at risk from visually feeding trout.

Under lighting conditions that mimicked either midday (high illumination) or dawn and dusk (low illumination), we specifically tested whether *P. media* and *A. capitata* (1) experienced differential rates of attack while on substrates which were either solidly coloured or patterned. These substrates were used, as they were typical in appearance to those found in the field and because the behaviour and vulnerability of stoneflies to trout can change depending on the appearance of the substrate (Feltmate and Williams 1989b). We also tested whether each stonefly (2) selected substrate, based on appearance, on which it was least vulnerable to predators and (3) altered periods of peak daytime activity (i.e. frequency of crawling) to minimize the impact of predators.

Methods

All nymphs used in these experiments were second year individuals (head width >3.5 mm) hand-picked from cobble substrates in Duffin Creek, southern Ontario (44°49'N, 79°08'W). Large nymphs were used because trout typically feed more on larger prey (Werner et al. 1983; Allan 1984). In the laboratory, nymphs were housed individually in large aerated containers and were fed enchytraeid whiteworms ad libitum. It was necessary to run all experiments in the laboratory, as detailed observations of the behaviour of stoneflies and trout, particularly under dim lighting conditions, could not be made in the field. As has been demonstrated in previous studies (Feltmate et al. 1986; Feltmate and Williams 1989a, 1989b), confinement in the laboratory does not have an adverse effect on the activity, foraging, and substrate selection behaviour of percid stoneflies.

We initially tested whether the vulnerability of *A. capitata* and *P. media* differed when they occurred on mottled or darkened substrates, under conditions of high (3.6×10^{15} quanta $\cdot$ s $^{-1}$ ·cm $^{-2}$) and low (8.0×10^{13} quanta $\cdot$ s $^{-1}$ ·cm $^{-2}$)

illumination. These levels of illumination approximated midday and dawn measurements, respectively, which we made in Duffin Creek 5.0 cm below the water surface. Commercial, unglazed tiles were used to construct cube-shaped substrates (5 $\times$ 5 $\times$ 5 cm) which were held together from the inside with silicon cement. The substrates were either completely dark brown or mottled in appearance, which represented typical colour regimes of substrates found in Duffin Creek and those for which we predicted the highest degree of crypsis for *P. media* and *A. capitata*, respectively. We constructed substrates, rather than use those found in Duffin Creek, to control for differences in surface texture that could potentially affect the behaviour of nymphs. For details of the classification of tile colours, see Feltmate and Williams (1989b). We constructed a rubber stamp that would print the identical size and pattern of mottling present on late-instar nymphs of *A. capitata*. The stamp was used to print dark patterns of the nymph, contiguously, on all surfaces of the cubes, thus producing the mottled effect. We applied the mottling pattern to cubes which, initially, were either light tan (hereafter referred to as light-mottled cubes) or medium brown (medium-mottled cubes) in colour. Two types of mottled cube were created so as to allow us to determine whether the vulnerability of stoneflies to trout would change relative to the reflectivity of the background (i.e. light tan versus medium brown) on which the mottling occurred. Previous experiments indicated that the ink used in marking did not affect behaviour of the nymphs (Feltmate and Williams 1989b).

Two light-mottled substrates were placed in an aquarium under conditions of high illumination. Each aquarium (30 cm long $\times$ 20 cm wide $\times$ 15 cm deep) was constructed from opaque, tan-coloured plastic and was roughened on the bottom with coarse sandpaper to enable the nymphs to crawl. The aquaria were filled to a depth of 13 cm and each was equipped with an air-stone to aerate and circulate the water. One 8-cm-long trout was placed in each aquarium, where it was restrained by a cylindrical mesh (800 μ m) enclosure. Trout this size have been shown to approach and sometimes bite larger stoneflies, but not consume them (Feltmate and Williams 1989a). Hence, we assumed that the nymphs would perceive the trout as a threat. We released one *P. media* nymph into each aquarium. Following a 20-min acclimation period, we removed the mesh cylinder and recorded the number of times the trout bit the stonefly over a subsequent 30-min period. Preliminary trials indicated that the attack frequency by trout did not diminish over 30 min; hence, no habituation by trout was apparent. At the end of the trial the stonefly was removed and the next trial began with a new nymph. This procedure was repeated 14 times for each of the six combinations of lighting condition and substrate type, with both *P. media* and *A. capitata*.

To examine substrate colour selection by stoneflies, under conditions of high and low illumination, we placed one light-mottled and one dark-brown cube at opposite ends in an aquarium (medium-mottled cubes were not used, as there was no significant difference in the number of attacks by trout on either species of stonefly when they occurred on light or medium mottled cubes). One trout, 8 cm long, was released into each aquarium on day 1. At 12:00 on day 2, one nymph was released into the aquarium, and 4 h later, we recorded the colour of the cube on which the nymph was located. Only one nymph was released per aquarium to preclude the possibility that mutual interference might affect selection. Previous experiments (Feltmate and Williams 1989a) established that 4 h was ample time for selection to occur. This procedure was repeated 20 times for each species of stonefly, each time using a different nymph.



FIG. 1. Mean percentage of time (± 1 SE) nymphs of *P. media* and *A. capitata* spent crawling, on either mottled (open) or dark-brown (hatched) substrate, under lighting conditions that simulated night (red light), dawn (low illumination), or day (high illumination), in the presence and absence of trout.

The activity of nymphs was recorded as the percentage of time individuals spent crawling under conditions of either (1) high or low illumination, (2) presence or absence of trout, and (3) light-mottled or dark-coloured substrate in aquaria. Three light-mottled cubes, or three dark cubes, separated by a distance of 8 cm each, were positioned longitudinally in each aquarium. In trials in which trout were present, one fish 8 cm in length was released at 12:00 on day 1. At 12:00 on day 2, one nymph was released in each aquarium and it was allowed a 20-min rest period before a trial commenced. Following acclimation, we recorded the time intervals during which the nymphs crawled over a 20-min period. For trials conducted in the absence of trout, exactly the same protocol was followed. This procedure was performed under lighting conditions of full illumination, low illumination, and darkness (i.e. red light, 1.1×10^{13} quanta $\cdot$ s $^{-1}$ $\cdot$ cm $^{-2}$). Red light was used to mimic darkness, as insects are not sensitive to light within this spectral range (Chapman 1982). Each of the 12 conditions was repeated five times, each time with a different nymph.

Results

The mean number of attacks by trout on *A. capitata* (mottled) and *P. media*, under conditions of high and low illumination and on substrates which were either light-mottled, medium-mottled, or dark brown, was normally distributed (Kolmogorov-Smirnov goodness of fit, $P > 0.05$ in all cases) and variance between conditions was homoscedastic (F -max, $P > 0.05$ in all cases). Two-way analysis of variance

(ANOVA) indicated a significant effect of light intensity ($P < 0.05$ for both species) and substrate type ($P < 0.05$ for both species), but no interaction between light intensity and substrate type ($P > 0.05$), on the number of attacks by trout on *A. capitata* and *P. media* (Table 1). Both species experienced fewer attacks when they occurred on the dark substrate as compared with the number experienced when nymphs were on either of the light- or medium-mottled substrates. The number of attacks on stoneflies was not significantly different between the two mottled substrate types (t -test, $P > 0.05$ in all cases). *Agneta capitata* experienced fewer attacks under low illumination and *P. media* under high illumination.

During the 20 substrate selection trials, both species showed significant preference for dark substrate over light-mottled substrate (*A. capitata*, 5 mottled, 15 dark, chi-square, $P < 0.05$; *P. media*, 2 mottled, 18 dark, $P < 0.001$).

The mean percentage of time *A. capitata* and *P. media* spent crawling, under all combinations of lighting condition and substrate type, was normally distributed (Kolmogorov-Smirnov, $P > 0.05$ in all cases) and variance between conditions was homoscedastic (F -max, $P > 0.05$ for both species). Three-way ANOVA indicated a significant effect of lighting intensity ($P < 0.05$ for both species), but no effect of substrate type ($P > 0.05$ for both species), nor significant interactions between lighting intensity, substrate type, or the presence of trout ($P > 0.05$ in all cases, for both species), on the percentage of time nymphs spent crawling (Fig. 1). *Agneta capitata* spent more time crawling under conditions of low illumination than under high

or red light illumination. *Paragnetina media* was most active during high illumination and least active under low illumination and red light conditions.

Discussion

The gross morphology and behavioural patterns of prey may function in tandem to minimize risk from predators (Harvey and Greenwood 1978; Sih 1987; Lima and Dill 1990). Our case supports this assertion, using rainbow trout and the stoneflies *A. capitata* and *P. media*. The mottled stonefly *A. capitata* was most active during periods of low illumination whereas the solidly coloured stonefly *P. media* was most active during periods of high illumination. The timing of these activities coincided with periods when lighting conditions rendered each species least vulnerable to attack by rainbow trout; thus, the behaviour functioned as a predetection or primary defence mechanism (i.e. the presence of the predator was not necessary to elicit the response; Edmunds 1974).

Unexpected in this study was, firstly, a reduced number of attacks by trout when *A. capitata* was on dark-brown rather than light- or medium-mottled substrate. However, as we demonstrated, the reduction in the number of attacks by trout was not related to changes in the frequency of movement of nymphs when they were on either dark or light-mottled substrate. Secondly, it was surprising that when given a choice between dark and light-mottled substrate, *A. capitata* selected the dark substrate. Subtle differences in the transmission properties of water, the ambient light spectrum, and/or ripples on the water surface may have affected, either singly or in combination, the trout's ability to discern *A. capitata* on the dark substrate. As Endler (1990) has suggested, more research on the visual acuity of predators is needed.

Although not considered in this study, *A. capitata* and *P. media* may have selected dark substrate not only to avoid detection by trout, but also to avoid being detected by prey. The utility of predator crypsis has been demonstrated in predaceous larval odonates (Moum and Baker 1990).

Many studies, particularly those which focus on streams, demonstrate the importance of predators, usually fishes, in affecting invertebrate population and community dynamics (e.g. Schofield et al. 1988; Gilliam et al. 1989; Schlosser and Ebel 1989; Feltmate and Williams 1991). Each of these studies relates relatively short-term behavioural responses of prey (e.g. changes in feeding rate or substrate selection) to longer term costs resulting from stress imposed by predators. When *P. media* and *A. capitata* restrict their "window" of daytime activity to periods when they are least vulnerable to rainbow trout, the nymphs are selecting a "slow life-style" (sensu Sih 1987) antipredator response. The short-term costs of this behaviour would probably result in reductions in feeding by nymphs (Feltmate and Williams 1989a), which could result in long-term reductions in the condition and fecundity of adults (Feltmate and Williams 1991). These costs would be countered by the long-term benefit of avoiding predators, since, as Lima and Dill (1990) reminded us, "being killed greatly reduces future fitness."

In summary, we show that solid coloration and patterning of the stoneflies *A. media* and *A. capitata* function to limit the threat posed by rainbow trout under conditions of high and low illumination, respectively. Both stoneflies have evolved such that the timing of their peak daytime activity coincides with periods when nymphs are least vulnerable to trout and, furthermore, the presence of the predator is not necessary to elicit

this behaviour. Our findings support the suggestion that the ability of predators to perceive prey varies as a function of light intensity and the degree of solid coloration versus patterning of prey. We suggest that, if sought, many examples which demonstrate the relationship between prey coloration, activity patterns, and avoidance of visually feeding predators will be found.

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Larval *Anisakis simplex* (Nematoda: Ascaridoidea) Infection in the Musculature of Atlantic Cod, *Gadus morhua*, from Newfoundland and Labrador

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Larvae of *Anisakis simplex* were found in the flesh of Atlantic cod, *Gadus morhua*, collected in 21 regions around Newfoundland and Labrador during 1985–87. Prevalence and abundance increased with cod size and varied geographically; cod off Labrador had the fewest larvae whereas those from the south coast of Newfoundland were the most heavily infected. Most larvae (~95%) occurred in flesh surrounding the body cavity (napes), with a significantly higher percentage of the nematodes (~58%) in the flesh on the left side. In 505 cod in which all tissues were examined, 85.6, 10.8, and 3.5% of the *A. simplex* resided in the body cavity and viscera, napes, and fillets, respectively. Cod surveyed tended to have more *A. simplex* in the musculature than those from other areas off eastern Canada, but are lightly infected compared with most other Atlantic cod stocks. The examination method (candling combined with slicing) recovered, on average, 42% of the *A. simplex* present in the flesh; consequently, infection statistics reported here are underestimates. Numbers of *A. simplex* in cod off Labrador and eastern Newfoundland are similar to those observed during 1947–53, but the abundance of *A. simplex* appears to have increased among cod from NAFO Subdivision 3Pn.

Nous avons découvert des larves d'*Anisakis simplex* dans la chair de morues franches, *Gadus morhua*, capturées dans 21 régions autour de Terre-Neuve et du Labrador entre 1985 et 1987. La prévalence et l'abondance de ce parasite augmentaient proportionnellement à la taille de la morue et présentaient des variations géographiques; les morues franches capturées au large du Labrador avaient le moins de larves, tandis que celles qui provenaient de la côte sud de Terre-Neuve étaient le plus gravement infestées. La plupart des larves (approximativement 95 %) ont été retrouvées dans la chair entourant la cavité abdominale (parois abdominales), un pourcentage beaucoup plus grand de nématodes (approximativement 58 %) étant logés dans la chair du flanc gauche. Chez 505 morues franches dont on a examiné tous les tissus, 85,6 % des *A. simplex* étaient logés dans la cavité abdominale et les viscères, 10,8 % dans les parois abdominales et 3,5 % dans les filets. Les morues examinées avaient tendance à avoir plus d'*A. simplex* dans les muscles que celles des autres zones au large des côtes orientales du Canada, mais elles étaient relativement moins infestées que dans la plupart des autres stocks de morues franches. La méthode d'examen utilisée (le mirage conjugué au filetage) a permis de récupérer, en moyenne, 42 % des *A. simplex* présents dans la chair du poisson; les statistiques mentionnées dans le présent document relativement aux infestations sont donc des sous-estimations. Le nombre d'*A. simplex* trouvés chez les morues franches capturées au large du Labrador et à l'est de Terre-Neuve est semblable aux dénombrements de 1947 à 1953, mais il semble y avoir un accroissement de l'abondance d'*A. simplex* chez les morues provenant de la subdivision 3Pn de la NAFO.

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Larvae of the ascaridoid nematodes *Anisakis simplex* and *Pseudoterranova decipiens* are common parasites of several species of marine fish. In Atlantic Canadian fisheries, the presence of larval *P. decipiens* is perceived mainly as a chronic cosmetic problem by the fishing industry and removal of the unsightly nematodes costs the industry several million dollars per annum (Malouf 1986). However, both *P. decipiens* and *A. simplex* are also capable of infecting man. Whereas human cases of *P. decipiens* are largely asymptomatic (Little and Most 1973; Chitwood 1975; Margolis 1977), except in Japan (see Nagano 1989), human infection with *Anisakis* can be more serious (Oshima 1972; Ishikura and Namiki 1989).

Although larvae of these nematodes in fish products can easily be killed by thorough freezing or cooking (Gustafson 1953; Bier 1976; Bier et al. 1987; Food and Drug Administration 1987; Deardorff and Throm 1988), in North America the num-

ber of cases of anisakiasis is increasing (Deardorff et al. 1987; McKerrow et al. 1988). This increase may be due to a greater awareness of the disease among the medical profession (McKerrow et al. 1988; Schantz 1988) combined with changing dietary habits that include increased consumption of raw, marinated, or lightly cooked seafood. In light of these findings, there is a need for information on the distribution and abundance of these nematodes in fish stocks that are harvested for human consumption.

In Canadian Atlantic waters, parasitological surveys have documented the occurrence of larval *A. simplex* in several major fisheries, including those for Atlantic salmon (*Salmo salar*) (Beverley-Burton and Pippy 1977), capelin (*Mallotus villosus*) (Palsson and Beverley-Burton 1984), Atlantic herring (*Clupea harengus*) (Parsons and Hodder 1971; McGladdery 1986), Atlantic cod (*Gadus morhua*), and American plaice (*Hippo-*

glossoides platessoides) (McClelland et al. 1983a, 1983b, 1985, 1987). The fishery for cod is by far the largest in Atlantic Canada, with total Canadian landings of ~461 000 metric tons during 1988. Although most (65%) of the total catch of cod is harvested off Newfoundland and Labrador, these stocks have not been surveyed for larvae of *A. simplex* in detail since the 1950's (Templeman et al. 1957). The objective of the present study was to determine the distribution and abundance of larval *A. simplex* in the flesh of cod around Newfoundland and Labrador and to compare the results with the previous survey. The occurrence of larval sealworm, *P. decipiens*, in these samples was described in a previous publication (Bratley et al. 1990).

Materials and Methods

Sampling

Samples of cod were collected during 1985–87 on Department of Fisheries and Oceans (DFO) research vessel cruises and from the commercial otter trawl fishery. Cod were taken from numerous locations in NAFO Divisions 2H, 2J3KL, and 3NO and Subdivisions 3Pn and 3Ps (Fig. 1).

All specimens, except for one batch of 107 (see below), were gutted at sea soon after capture. Most specimens from research cruises were frozen (-20°C) prior to examination whereas commercial samples were placed on ice and generally examined fresh. Gutted fish were weighed (nearest gram) and the fork length measured (nearest centimetre). The methodology employed during examination of the flesh was similar to that used by Templeman et al. (1957) and McClelland et al. (1983a, 1983b) and is described in detail elsewhere (Bratley et al. 1990). Briefly, flesh on the left and right sides of the fish was removed and fillets separated from napes (=belly flap or hypaxial musculature) by cutting where the ribs join the spinal cord and extending the cut posteroventrally to a point approximately at the middle of the anal fin. Left and right fillet and nape from each fish were skinned, weighed (nearest 0.1 gm), and examined for nematodes on a candling light (Maritime Plastics, Donovan's Industrial Estate, St. John's, Nfld.). The musculature of all but the smallest fish (<40 cm) was sliced diagonally into thin strips during examination to reveal nematodes deeply embedded in the flesh, and the approximate position of nematodes was recorded on a grid (Fig. 2). Nematodes were preserved in a 1:9 solution of glycerin – 70% ethyl alcohol.

To determine the proportion of nematodes recovered by our examination procedures, the flesh of 107 cod (mean fork length $\pm$ SD = 57.6 ± 8.3 cm, range 42–107 cm) collected in NAFO Subdivision 3Ps was reinspected using a mechanical disintegration technique which recovers nematodes undetected during candling and slicing and recovers close to 100% of the nematodes present (Bratley 1988). This procedure indicated that percentage recovery for *A. simplex* during candling and slicing was 42.1%. The proportion recovered was, therefore, comparable with that reported by McClelland et al. (1983a) for *A. simplex* in cod from other regions off eastern Canada.

An additional sample of 505 gutted cod was collected from Fortune Bay during January 1987 and examined to determine the total numbers of *A. simplex* present and the proportions occurring in various tissues (fillets, napes, liver, and other viscera). These fish were frozen immediately after capture. The mean fork length of the fish $\pm$ SD was 52.5 ± 4.7 cm (range 39–64). Pepsin – hydrochloric acid (HCl) digestion (Stern et al. 1958) followed by inspection under ultraviolet light (see Bratley 1988) was used to aid in recovery of nematodes from the flesh

and viscera; this technique also recovers close to 100% of the nematodes present. Fillets, napes, liver, and other viscera were digested and examined separately to determine the numbers of *A. simplex* in each of these tissues.

Taxonomy

Detailed morphological examination of several dozen of our specimens under a compound microscope indicated that they closely resembled *A. simplex* larva (I) of Berland (1961); however, Nascetti et al. (1986) and Orecchia et al. (1986) recently showed, using genetic evidence based on allozyme electrophoresis, that *A. simplex* consisted of two sibling species provisionally designated *A. simplex* A and B. Whereas Type A occurred mainly in the Mediterranean, Type B was widely distributed in the Northeast Atlantic. To confirm the identification of the nematodes found here, we sent several specimens from cod and other fish species to Prof. L. Paggi and co-workers at the University of Rome, "La Sapienza", Rome, Italy, for analysis using allozyme electrophoresis (for details, see Nascetti et al. 1986). Their findings indicated that all specimens were *A. simplex* B. We conclude, therefore, that *A. simplex* B is probably the common species infecting cod and other fishes around Newfoundland and Labrador, but these findings do not exclude the possibility that other species of *Anisakis* also occur in this area (e.g. see Threlfall 1982).

Statistical Methods

Terminology for nematode parasite infection statistics follows standard usage (Margolis et al. 1982): prevalence = proportion of cod infected expressed as a percentage; abundance = mean number of *A. simplex* per cod including uninfected fish; density = mean number of *A. simplex* per kilogram of flesh including uninfected fish.

Since our survey utilized trawling as a sampling method, with several fish being taken from each of several sets within an area, our fish were obtained using a clustered rather than random sampling method. Consequently, standard statistical methods which assume random sampling cannot be used to analyze the parasite data because there may be heterogeneity in the proportion of infected fish within an area. To alleviate this problem, we used methods similar to those described in Bratley et al. (1990). In the analysis of binary data, such as where fish are assigned to one of two classes (i.e. infected or not infected), it is usually assumed that the number of fish in a class, e.g. those infected, follows a binomial distribution. However, this assumption may not be valid if there is significant heterogeneity in the proportion of infected fish within a sampling region. To analyze this heterogeneity, we assumed that in any one trawl, the number of infected cod followed a binomial distribution with parameter p and that p varied as a beta distribution with parameters α and β . The variation in the proportion of cod infected therefore follows a beta-binomial distribution (Paul and Plackett 1978). Within a sample, if there were n fish sampled from m trawls, then the variance in the proportion infected is

$$\frac{p(1-p)(n\epsilon + 1)}{m\epsilon(\epsilon + 1)}$$

where $\epsilon = 1/(\alpha + \beta)$. Therefore, the variance for the beta-binomial distribution is the heterogeneity factor $(n\epsilon + 1)/(\epsilon + 1)$ times the variance of the beta-binomial distribution (Paul and Plackett 1978). Heterogeneity factors were computed using the mean sample size per trawl, $\bar{n}$ (Table 1). Maximum

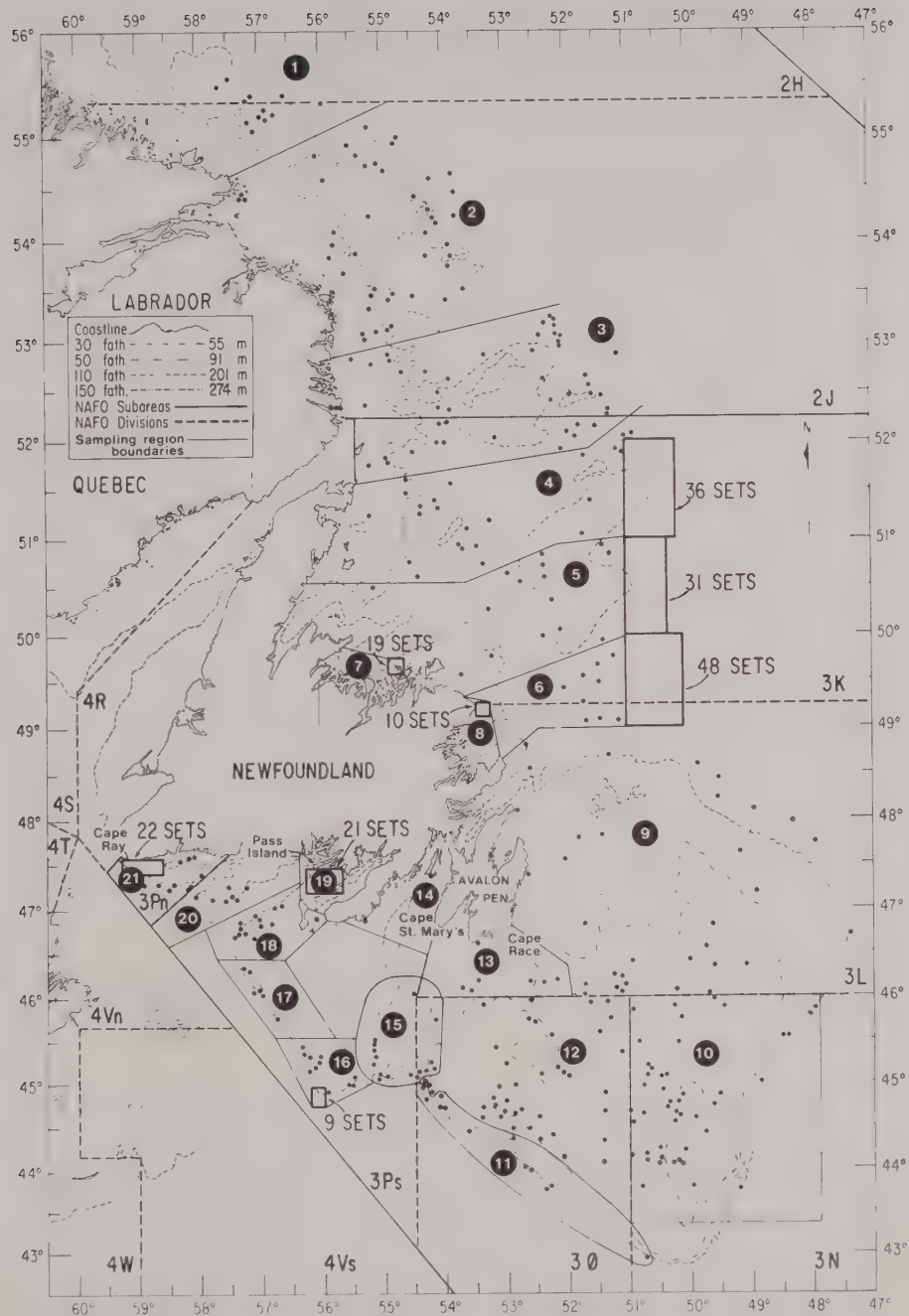


FIG. 1. Waters off eastern Canada showing NAFO divisions and trawl locations (dots) from which samples of cod were obtained. Rectangles indicate boundaries of intensively sampled areas. Solid lines indicate boundaries of the 21 sampling regions (1 = Makkovik Bank, 2 = Hamilton Inlet Bank, 3 = Belle Isle Bank, 4 = northern Funk Island Bank, 5 = southern Funk Island Bank, 6 = northeast Newfoundland Shelf, 7 = Notre Dame Bay, 8 = Bonavista Bay, 9 = Grand Bank, 10 = southeast shoal of Grand Bank, 11 = southwest slope of Grand Bank, 12 = Whale Bank, 13 = southern Avalon, 14 = Placentia Bay, 15 = Green Bank, 16 = southern St. Pierre Bank, 17 = western St. Pierre Bank, 18 = northern St. Pierre Bank, 19 = Fortune Bay, 20 = Burgeo Bank, 21 = Rose Blanche Bank).

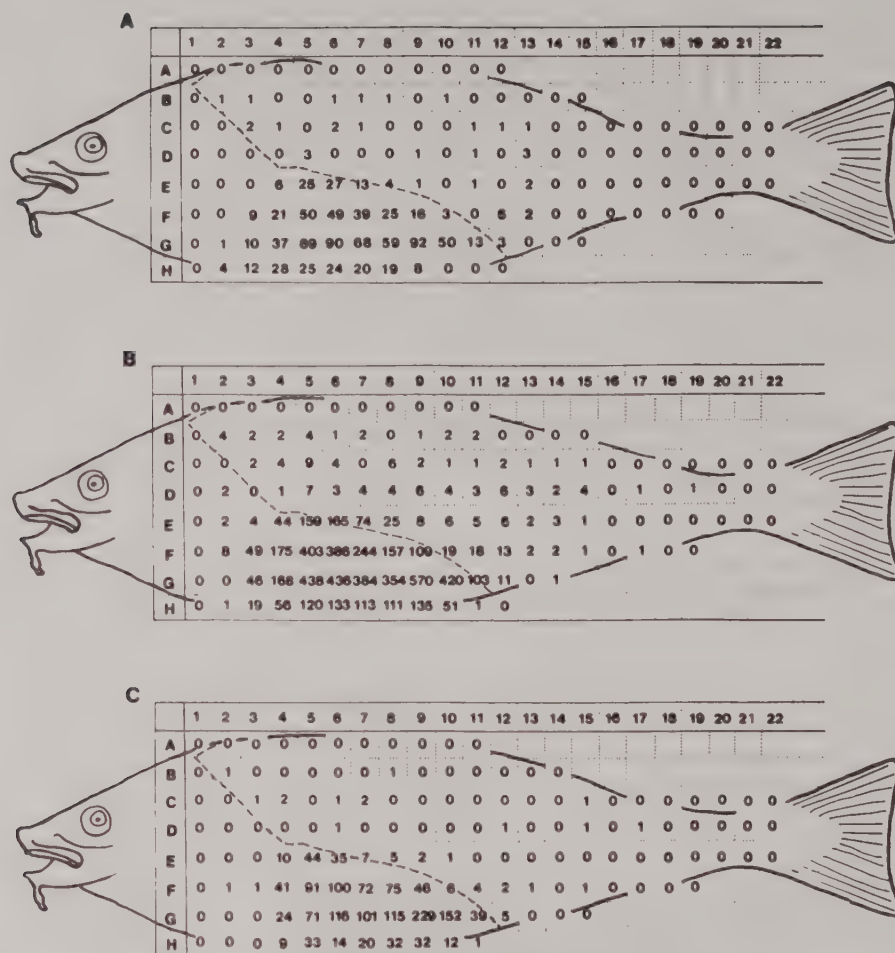


FIG. 2. Numbers of larval *A. simplex* recovered from various regions of the musculature of three size groups of Atlantic cod, from Newfoundland and Labrador. The broken line indicates the approximate location of the cut dividing the fillet and nape portions. (A) ≤ 50 cm, $N = 3465$; (B) 51–70 cm, $N = 7416$; (C) ≥ 70 cm, $N = 1676$ (where N = number of fish examined).

likelihood estimates of α and β were obtained for each sample using a quasi-Newtonian algorithm. The number of fish examined can be divided by the heterogeneity factor to adjust the sample size to the value needed for meaningful statistical tests (Griffiths 1973).

We computed heterogeneity factors for cod in each of three length classes from six general areas off Newfoundland and Labrador (Table 1); the areas were the same as those described in Bratley et al. (1990), except that one additional area (southern Grand Banks) is also included here. For samples collected off Labrador and the east coast of Newfoundland, there was little within-area heterogeneity, i.e. the heterogeneity factors were generally close to 1 and the assumption of random sampling is reasonable. The greatest heterogeneity in the proportion of infected fish was among samples of small (31–50 cm) and medium-sized (51–70 cm) cod collected off the south coast of Newfoundland and medium-sized cod from the southern Grand Bank, where heterogeneity factors ranged from 2.01 to 3.78. Thus, for these areas, considerable adjustment of the sample sizes would be needed before valid statistical comparisons of prevalence could be made.

Results

Geographic Distribution

Larvae of *A. simplex* were found in the flesh of cod of a wide range of sizes in all 21 regions surveyed (Table 2; Fig. 1). In most regions, the prevalence and abundance of *A. simplex* tended to increase with cod length, often reaching a maximum in the 70–79 cm group and declining thereafter. The methods employed here (candling combined with slicing) recover only about 42% of the total number of *A. simplex* in the flesh; consequently, the infection levels are higher than those reported in Table 2. Nonetheless, the results indicate that the nematode is common and widely distributed in cod around Newfoundland and Labrador. Cod from the most northerly areas (Makkovik Bank, Hamilton Inlet Bank, Belle Isle Bank, northern Funk Island Bank) had the lowest infection levels (with overall prevalences ranging from 9.9 to 25.3% and abundances from 0.11 to 0.24 nematodes/fish) whereas cod from areas off the south coast of Newfoundland (southern St. Pierre Bank, Fortune Bay, Rose Blanche Bank) were the most heavily infected (53.4–66.5% and 1.05–1.74 nematodes/fish). In general, small

TABLE 1. Heterogeneity factors for the proportions of cod infected with *A. simplex* in the musculature. $\bar{n}$ = mean sample size per trawl, α and β are parameters of the beta-binomial distribution, and $\epsilon = 1/(\alpha + \beta)$.

Sampling area	Cod length class (cm)	Number of trawls	$\bar{n}$	α	β	ϵ	Heterogeneity factor
Newfoundland east coast (inshore)	31–50	24	27.9	118.2	537.8	0.002	1.041
	51–70	23	33.6	15.8	29.1	0.022	1.710
	71–150	10	8.0	284.5	255.2	0.002	1.013
Labrador (inshore and offshore)	31–50	52	12.9	4.2	39.4	0.023	1.267
	51–70	54	11.1	16.9	67.7	0.012	1.118
	71–150	52	2.3	2.0	5.5	0.133	1.149
Newfoundland south coast (Cape Race to Pass Island)	31–50	23	11.7	2.5	4.8	0.136	2.281
	51–70	23	36.8	6.6	5.3	0.084	3.782
	71–150	20	9.3	49.9	32.2	0.012	1.099
St. Pierre Bank	31–50	14	8.1	57.0	532.7	0.002	1.012
	51–70	19	11.3	4.7	7.1	0.085	1.806
	71–150	22	7.3	346.3	478.2	0.001	1.008
Newfoundland south coast (Pass Island to Cape Ray)	31–50	25	9.5	3.3	3.4	0.150	2.114
	51–70	27	15.1	4.9	2.1	0.143	2.758
	71–150	20	5.3	2.9	2.0	0.204	1.730
Southern Grand Bank	31–50	48	5.1	1.9	12.4	0.070	1.268
	51–70	55	11.3	3.3	5.8	0.109	2.012
	71–150	118	4.5	3.8	6.0	0.102	1.328

numbers of *A. simplex* were recovered from the flesh of individual fish; the maximum recovered was 13. On the basis of nematodes per unit weight of flesh (number of *A. simplex*/kg), cod in most areas were lightly infected overall (≤ 0.99 nematodes/kg), but in many areas, some individual size groups had high densities (≥ 1.00) of *A. simplex* (e.g. 60–69 cm fish from northeast Newfoundland Shelf, Notre Dame Bay, Grand Bank, Green Bank, Placentia Bay). The areas where overall densities were highest (1.17–2.29) were southern St. Pierre Bank, Fortune Bay, and Rose Blanche Bank. The relationship between nematode density and cod length was variable but tended to show a peaked pattern, often with the highest densities (> 1.00 worm/kg) among medium-sized (50–70 cm) fish.

Comparison with Previous Survey

Templeman et al. (1957) provided data on the numbers of nematodes and the proportions of *Anisakis* in the fillets (i.e. excluding nape portion) of cod ≥ 41 cm in length collected around Newfoundland and Labrador during 1947–53. From this historical information, we calculated the abundance (mean number of worms) and density (worms per kilogram) of *A. simplex* in the fillets of cod and compared his results with those obtained here for 11 of the sampling areas defined in table IV in Templeman et al. (1957). Detailed statistical analyses of these data were not possible because Templeman et al. did not provide estimates of variance around the mean. Both the abundance and density of *A. simplex* were low (≤ 0.19 and ≤ 0.32 , respectively) in all areas during both surveys (Table 3). The data suggest no major changes in the numbers of *A. simplex* in the fillets of cod from Labrador, the Grand Banks, and the east and northeast coasts of Newfoundland. Along the south coast of Newfoundland, trends in the abundance and density of *A. simplex* appear to be more variable. However, the significance of differences for some areas should be treated with caution; Templeman et al.'s data for the proportions of *A. simplex* and *P. decipiens* were often based on examination of small numbers (≤ 16) of nematodes (see Table 3); consequently, for

areas such as the Grand Banks, the east and northeast coasts of Newfoundland, St. Pierre Bank south of 46°30'N, and the south coast of Newfoundland between Cape Race and Cape St. Mary's, the estimates of abundance and density of *A. simplex* for 1947–53 may be more prone to variation. Also, differences in the size composition of the samples between surveys and the higher heterogeneity in the infection among cod from the south coast of Newfoundland (see Table 1) could also influence the validity of this comparison. Nonetheless, current abundances and densities appear to be higher among cod collected from Cape St. Mary's to Pass Island and Pass Island to Cape Ray (3Pn) but lower among samples from northern St. Pierre Bank and Burgeo Bank.

Distribution in Cod Tissues

Most larvae of *A. simplex* were concentrated in the napes, particularly near the vent (grid squares G10 and G11; Fig. 2); some had migrated deeply into the fillets (i.e. D17, D19) but these were rare. There were no distinct changes in the distribution of larval *A. simplex* in the flesh of cod with size; the slightly greater total numbers of nematodes recovered from the fillets of medium-sized fish (51–70 cm) compared with other size groups can be attributed to the much higher sample size ($N = 7416$) and higher abundance of nematodes among the medium-sized cod (Table 2).

The distribution of *A. simplex* in the left and right fillets and napes was similar in cod of a wide range of sizes from two stock complexes (Table 4). In most size groups, more nematodes were recovered from the left fillet and nape although the differences were significant ($P < 0.05$) mainly for the nape portion of each size class. Fewer nematodes were recovered from fillets, and differences between left and right sides were not significant, except for the pooled data for fillets of cod from 3P ($G = 6.48$, $P < 0.05$). Values for heterogeneity G were not significant ($P > 0.05$), indicating little difference among cod size classes in the proportion of nematodes recovered from left versus right sides. The percentage of the overall totals (i.e.

TABLE 2. Summary of infection statistics for *A. simplex* in the musculature of Atlantic cod collected off Newfoundland and Labrador during 1985–87. Prevalence = percentage of fish infected; abundance = mean number of *A. simplex* per cod including uninfected fish; maximum = maximum number of *A. simplex* in a single cod; density = mean number of *A. simplex* per kilogram of flesh (including uninfected fish). For sampling areas, see Fig. 1.

NAFO Div.	Sampling area	Dates	Length group (cm)	Number of fish	Prevalence	Abundance, variance	Maximum	Density
2H, 2J	1. Makkovik Bank	Oct. 1985; Sept., Nov. 1986	<30	10	10.00	0.10, 0.10	1	1.51
			30–39	21	0.00	0.00, 0.00	0	0.00
			40–49	33	9.09	0.09, 0.09	1	0.30
			50–59	41	7.32	0.10, 0.14	2	0.19
			60–69	17	23.53	0.29, 0.35	2	0.35
			70–79	10	20.00	0.20, 0.18	1	0.13
			Total	132	9.85	0.11, 0.13	2	0.23
2J	2. Hamilton Inlet Bank	Oct., Nov. 1985; Aug.–Nov. 1986	30–39	81	2.47	0.02, 0.02	1	0.15
			40–49	110	7.27	0.10, 0.16	3	0.34
			50–59	137	12.41	0.18, 0.30	3	0.34
			60–69	40	25.00	0.35, 0.59	4	0.43
			70–79	31	22.58	0.32, 0.43	2	0.26
			80–89	17	11.76	0.12, 0.11	1	0.06
			≥90	26	34.62	0.50, 0.74	3	0.15
			Total	442	12.44	0.17, 0.28	4	0.25
2J, 3K	3. Belle Isle Bank	Oct., Nov. 1985; Aug., Nov., Dec. 1986; Feb.–Mar. 1987	<30	23	0.00	0.00, 0.00	0	0.00
			30–39	34	2.94	0.03, 0.03	1	0.16
			40–49	350	12.86	0.15, 0.19	3	0.34
			50–59	366	21.58	0.30, 0.42	4	0.44
			60–69	67	22.39	0.40, 0.70	3	0.41
			70–79	38	31.58	0.50, 1.23	6	0.40
			80–89	23	17.39	0.17, 0.15	1	0.09
			≥90	18	22.22	0.39, 0.72	3	0.10
3K	4. Northern Funk Island Bank	Nov. 1985; Jan., Mar., Nov.–Dec. 1986; Feb. 1987	30–39	17	11.76	0.18, 0.28	2	0.77
			40–49	382	14.40	0.20, 0.33	4	0.46
			50–59	601	29.28	0.49, 1.00	8	0.73
			60–69	154	35.71	0.62, 1.15	5	0.63
			70–79	31	32.26	0.61, 1.25	4	0.40
			80–89	16	37.50	0.63, 0.92	3	0.29
			≥90	15	26.67	0.67, 1.67	4	0.17
			Total	1216	25.33	0.42, 0.83	8	0.58
3K	5. Southern Funk Island Bank	Nov. 1985; Jan.–Mar., Sept., Nov. 1986	40–49	172	22.09	0.28, 0.37	4	0.68
			50–59	758	33.38	0.57, 1.23	7	0.94
			60–69	335	49.85	0.90, 1.72	8	0.96
			70–79	81	49.38	0.86, 1.37	5	0.62
			80–89	21	38.10	0.71, 1.11	3	0.35
			Total	1367	37.02	0.63, 1.28	8	0.87
3K, 3L	6. Northeast Nfld. Shelf	Nov. 1985; Jan.–Mar., Nov. 1986; Feb. 1987	30–39	13	0.00	0.00, 0.00	0	0.00
			40–49	246	19.11	0.26, 0.39	4	0.61
			50–59	851	44.42	0.73, 1.25	10	1.18
			60–69	406	53.69	1.03, 1.93	11	1.11
			70–79	95	65.26	1.23, 2.41	10	0.87
			80–89	17	35.29	0.65, 0.99	3	0.31
			≥90	17	29.41	0.41, 0.63	3	0.11
			Total	1645	43.53	0.75, 1.41	11	0.99
3K	7. Notre Dame Bay	July–Sept. 1986	40–49	369	20.33	0.23, 0.24	3	0.64
			50–59	371	34.23	0.47, 0.56	4	0.86
			60–69	142	50.00	0.82, 1.31	6	1.03
			Total	882	30.95	0.43, 0.59	6	0.84

TABLE 2. (Continued)

NAFO Div.	Sampling area	Dates	Length group (cm)	Number of fish	Prevalence	Abundance, variance	Maximum	Density
3L	8. Bonavista Bay	Aug.–Sept. 1986	40–49	172	12.79	0.14, 0.14	2	0.43
			50–59	178	20.22	0.26, 0.34	4	0.48
			60–69	105	42.86	0.59, 0.69	4	0.69
			70–79	29	58.62	0.69, 0.44	2	0.59
			Total	484	24.79	0.31, 0.39	4	0.55
3L	9. Grand Bank	Feb.–Mar., Aug.–Sept., Nov. 1986	30–39	29	3.45	0.03, 0.03	1	0.22
			40–49	70	10.00	0.10, 0.09	1	0.31
			50–59	178	39.33	0.66, 1.42	9	1.16
			60–69	122	57.38	1.17, 2.18	6	1.32
			70–79	86	46.51	0.92, 1.56	6	0.68
			80–89	32	46.88	0.59, 0.51	2	0.30
			≥90	35	31.43	0.43, 0.49	2	0.11
			Total	552	38.77	0.69, 1.38	9	0.70
3N	10. Southeast Shoal of the Grand Bank	May–Sept. 1985; Apr.–May 1986	30–39	19	5.26	0.05, 0.05	1	0.36
			40–49	20	5.00	0.05, 0.05	1	0.17
			50–59	22	31.82	0.41, 0.44	2	0.79
			60–69	20	25.00	0.25, 0.20	1	0.31
			70–79	27	25.93	0.41, 0.71	3	0.31
			80–89	27	18.52	0.26, 0.43	3	0.14
			≥90	115	33.04	0.50, 0.76	5	0.11
			Total	250	25.60	0.36, 0.55	5	0.14
30, 3N	11. Southwest Slope of the Grand Bank	Apr. 1985; Jan., Mar.–Apr. 1986; Jan.–Mar. 1987	40–49	15	33.33	0.33, 0.24	1	1.13
			50–59	96	20.83	0.23, 0.22	2	0.41
			60–69	71	38.03	0.56, 0.96	5	0.63
			70–79	31	64.52	1.45, 2.26	5	1.12
			80–89	28	60.71	1.39, 2.84	6	0.70
			≥90	10	20.00	0.30, 0.46	2	0.12
			Total	251	36.25	0.61, 1.18	6	0.63
3O	12. Whale Bank	Apr. 1985; Mar.–Apr., Nov. 1986; Feb. 1987	30–39	31	9.68	0.10, 0.09	1	0.84
			40–49	29	13.79	0.14, 0.12	1	0.56
			50–59	55	14.55	0.18, 0.23	2	0.36
			60–69	63	41.27	0.67, 1.03	5	0.83
			70–79	36	41.67	0.83, 1.69	5	0.66
			80–89	27	59.26	0.96, 1.04	3	0.55
			≥90	102	40.20	0.88, 2.03	7	0.20
			Total	343	32.94	0.60, 1.20	7	0.33
3L	13. Southern Avalon	Feb., Sept., Nov. 1986; Feb. 1987	30–39	28	0.00	0.00, 0.00	0	0.00
			40–49	46	17.39	0.24, 0.32	2	0.76
			50–59	70	22.86	0.29, 0.35	3	0.49
			60–69	63	33.33	0.54, 0.99	5	0.62
			70–79	34	67.65	1.18, 1.30	5	0.88
			80–89	27	81.48	1.96, 2.42	6	0.92
			≥90	36	61.11	1.17, 3.23	9	0.27
			Total	304	36.84	0.66, 1.37	9	0.54
3Ps	14. Placentia Bay	Sept.–Oct. 1986	30–39	22	4.55	0.05, 0.05	1	0.28
			40–49	57	14.04	0.16, 0.17	2	0.52
			50–59	56	35.71	0.57, 0.94	4	1.02
			60–69	57	47.37	1.04, 2.28	6	1.14
			70–79	49	65.31	1.14, 1.50	6	0.87
			80–89	19	57.89	1.21, 2.62	6	0.63
			≥90	13	23.08	0.31, 0.40	2	0.07
			Total	273	37.36	0.67, 1.34	6	0.71

TABLE 2. (Concluded)

NAFO Div.	Sampling area	Dates	Length group (cm)	Number of fish	Prevalence	Abundance, variance	Maximum	Density
3Ps, 3O	15. Green Bank	Feb.–Apr. 1986; Feb.–Mar. 1987	40–49	38	21.05	0.29, 0.37	2	0.71
			50–59	250	32.80	0.52, 0.84	5	0.89
			60–69	189	49.21	0.96, 1.80	9	1.09
			70–79	50	80.00	1.62, 2.89	8	1.14
			80–89	29	44.83	0.72, 1.06	4	0.34
			≥90	29	58.62	1.14, 2.05	6	0.33
			Total	585	43.25	0.78, 1.48	9	0.82
3Ps	16. Southern St. Pierre Bank	Jun.–Mar. 1986; Jan.–Mar. 1987	40–49	15	26.67	0.27, 0.21	1	0.68
			50–59	171	41.52	0.66, 1.04	6	1.04
			60–69	145	62.07	1.22, 2.06	7	1.29
			70–79	59	81.36	1.98, 2.98	10	1.35
			80–89	9	55.56	0.78, 0.94	3	0.35
			Total	399	54.64	1.05, 1.88	10	1.17
3Pn	17. Western St. Pierre Bank	Jan.–Mar. 1986; Jan.–Feb. 1987	<30	12	0.00	0.00, 0.00	0	0.00
			30–39	17	5.88	0.06, 0.06	1	0.43
			40–49	21	9.52	0.14, 0.23	2	0.41
			50–59	80	40.00	0.58, 0.86	5	1.04
			60–69	45	51.11	0.93, 1.75	6	1.08
			70–79	40	57.50	1.10, 1.94	6	0.75
			80–89	29	51.72	1.48, 4.54	8	0.70
			≥90	38	26.32	0.50, 1.45	6	0.13
			Total	282	37.59	0.70, 1.62	8	0.55
3Ps	18. Northern St. Pierre Bank	Mar. 1985; Jan.–Mar. 1986; Jan.–Mar. 1987	<30	10	0.00	0.00, 0.00	0	0.00
			30–39	32	9.38	0.09, 0.09	1	0.72
			40–49	42	9.52	0.12, 0.16	2	0.46
			50–59	45	20.00	0.31, 0.49	3	0.65
			60–69	36	36.11	0.58, 0.76	3	0.82
			70–79	34	44.12	0.85, 1.40	4	0.77
			80–89	19	31.58	0.47, 0.71	3	0.27
			≥90	16	43.75	0.81, 1.36	4	0.24
			Total	234	24.36	0.40, 0.67	4	0.50
3Ps	19. Fortune Bay	Jan.–Mar. 1986; Jan.–Mar. 1987	40–49	85	34.12	0.65, 1.56	8	1.66
			50–59	451	50.11	1.00, 1.81	9	1.58
			60–69	275	61.45	1.36, 3.01	13	1.42
			70–79	88	63.64	1.60, 4.31	11	1.10
			Total	899	53.39	1.13, 2.46	13	1.44
3Ps	20. Burgeo Bank	Feb. 1987	30–39	13	7.69	0.08, 0.08	1	0.56
			40–49	16	12.50	0.13, 0.12	1	0.43
			50–59	17	17.65	0.24, 0.32	2	0.45
			60–69	18	33.33	0.61, 0.96	3	0.72
			70–79	12	25.00	0.42, 0.63	2	0.31
			80–89	12	33.33	1.00, 2.73	4	0.49
			Total	88	21.59	0.40, 0.79	4	0.49
3Pn	21. Rose Blanche Bank	Feb.–Mar. 1986; Feb.–Mar. 1987	30–39	18	16.67	0.44, 1.56	5	3.69
			40–49	155	53.55	1.15, 2.26	7	3.34
			50–59	317	70.66	1.71, 3.40	11	3.27
			60–69	94	81.91	2.83, 6.01	10	3.51
			70–79	25	84.00	3.00, 8.33	12	2.33
			80–89	19	73.68	1.79, 2.62	5	0.90
			≥90	32	53.13	1.44, 3.42	6	0.34
			Total	660	66.52	1.74, 3.94	12	2.29

TABLE 3. Temporal trends in the abundance (mean no. of worms/fish) and density (worms/kg fillet) of *A. simplex* in the fillets of Atlantic cod >41 cm in length from Newfoundland and Labrador. Values for 1947–53 were calculated using data from table IV in Templeman et al. (1957). Values in parentheses are numbers of nematodes examined by Templeman et al. and used to estimate the proportion of nematodes that were *A. simplex*.

Sampling area: NAFO Division	Number of cod examined		Abundance		Density	
	1947–53	1985–87	1947–53	1985–87	1947–53	1985–87
SE Grand Bank: 3N	1550	246	0.01 (7)	0.01	0.01	0.01
N. Grand Bank: 3L	1004	394	0.01 (8)	0.02	0.01	0.02
SW Grand Bank: 3O	922	786	0.02 (14)	0.03	0.03	0.05
NE coast of Newfoundland: 3K	699	4093	0.01 (3)	0.03	0.02	0.03
Labrador offshore: 2G, 2H, 2J	851	1212	0.02 (11)	0.01	0.05	0.01
East coast of Newfoundland inshore: 3K, 3L	1089	1494	0 (14)	0.01	0	0.02
St. Pierre Bank, south of 46°30'N: 3Ps	344	253	<0.01 (16)	0.04	0.01	0.04
South coast of Newfoundland, Cape St. Mary's to Pass Island: 3Ps	736	1142	0.01 (97)	0.05	0.01	0.08
South coast of Newfoundland, Cape Race to Cape St. Mary's: 3L	257	98	0.09 (7)	0.02	0.14	0.03
St. Pierre Bank, north of 46°30'N and Burgeo Bank: 3Ps	551	270	0.05 (109)	0.01	0.06	0.01
South coast of Newfoundland, Pass Island to Cape Ray: 3Pn	935	640	0.03 (362)	0.19	0.04	0.32

from both fillets and napes combined) occurring in the fillets was generally low (4.2 and 6.9%). *G*-tests of heterogeneity also revealed that the percentage recovered from the fillets did not differ significantly ($G = 3.58$, $P > 0.1$, $df = 5$) with size among 2J–3KL cod but appeared to decrease with size ($G = 32.13$, $P < 0.001$, $df = 5$) among those from 3P.

In the additional sample of 505 cod from Fortune Bay, in which all tissues (i.e. flesh and viscera) were examined, only 12.2–17.7% of the total numbers of *A. simplex* occurred in the flesh (fillets and napes combined; Table 5). These data also indicated that ~24.5% of the nematodes recovered from the flesh were in the fillet portion, compared with 1.9–9.6% in the fillets of cod examined by candling and slicing (Table 4). This comparison suggests that a greater proportion of the *A. simplex* in the thicker fillet portion is not detected during candling and slicing. In the pepsin–HCl digested samples, the majority (31.7–47.1) of the *A. simplex* were found either in the liver (usually on the surface) or among the other viscera (35.3–56.1%), particularly around the pyloric caeca. There were no distinct size-related changes in the distribution of *A. simplex* among the tissues of these cod, at least over the size range examined (40–60 cm). The nematode was particularly common in cod from Fortune Bay, with prevalence and abundance increasing with size and ranging from 65 to 92.9% and 1.7 to 5.0 worms/fish, respectively.

Discussion

Our survey demonstrates that larvae of *A. simplex* are common and widely distributed in cod stocks around Newfoundland

and Labrador. The numbers of *A. simplex* in the flesh are undoubtedly higher than those reported here (Table 2) because the methods employed during the survey (candling combined with slicing) recovered only about 42% of the *A. simplex* present. Nonetheless, our findings indicate that a large proportion of fish in many areas harbour *A. simplex* and that the numbers present in the flesh of individual fish are generally low.

Geographic trends in the prevalence and abundance of *A. simplex* in the flesh of cod are similar to those reported previously for larval sealworm, *P. decipiens* (Brattey et al. 1990). Cod from Labrador, eastern Newfoundland, and the Grand Banks are more lightly infected than those collected off the south coast. Heterogeneity factors are also higher for both *A. simplex* and *P. decipiens* among cod from areas off the south coast, indicating more within-area variability in the proportion of infected fish. Since the higher degree of heterogeneity may be due to mixing of cod stocks with different levels of infection, parasitological data appear consistent with the results of conventional tagging studies (Templeman 1974, 1979; Lear 1984) which show that the assemblage of cod stocks off southern Newfoundland are more heterogeneous than those off Labrador and the east and northeast coasts of Newfoundland. The most heavily infected fish, with respect to both *A. simplex* and *P. decipiens*, were found in winter samples from NAFO subdivision 3Pn, which probably included many cod from 4R which overwinter in 3Pn. The 4R cod are known to be heavily infected with both nematode species (McClelland et al. 1985).

Although comparison between the results of the present study and that of Templeman et al. (1957) should be treated with

TABLE 4. Distribution of larval *A. simplex* in the flesh (left and right fillet and nape) of various size classes of Atlantic cod collected off Newfoundland and Labrador during 1985–87. The flesh was examined by candling and slicing. Replicated goodness-of-fit tests employing the *G*-statistic were used to test the hypothesis that equal numbers of nematodes occur in the left and right sides of each size class of cod. ns = not significant ($P > 0.05$), *significant ($P < 0.05$), **significant ($P < 0.001$). Degrees of freedom are 1 for individual size groups and pooled *G*, 5 for heterogeneity *G*, and 6 for total *G*.

Cod stock complex	Length class (cm)	Number of cod	Nape				Fillet				% of total <i>A. simplex</i> in fillets
			Number of <i>A. simplex</i>	% in left side	<i>G</i>	<i>P</i>	Number of <i>A. simplex</i>	% in left side	<i>G</i>	<i>P</i>	
2J–3KL	40–49	1936	369	61.3	18.83	**	17	58.8	0.53	ns	4.4
	50–59	3559	1783	60.3	76.08	**	80	55.0	0.80	ns	4.3
	60–69	1446	1172	59.2	40.04	**	44	50.0	0	ns	3.6
	70–79	443	362	64.6	31.50	**	22	54.6	0.18	ns	5.7
	80–89	163	123	57.7	2.95	ns	4	75.0	1.05	ns	3.2
	>90	159	100	60.0	4.03	*	4	75.0	1.05	ns	3.9
	Total		3909	60.4	173.42	**	171	55.0	3.61	ns	4.2
				Pooled	169.49	**		Pooled	1.69	ns	
				Heterogeneity	3.94	ns		Heterogeneity	1.92	ns	
3P	40–49	434	255	56.1	3.78	ns	27	66.7	3.06	ns	9.6
	50–59	1317	1249	58.8	38.60	**	122	58.2	3.29	ns	8.9
	60–69	796	1073	58.6	32.06	**	72	55.6	0.89	ns	6.3
	70–79	352	525	57.7	12.55	**	20	60.0	0.81	ns	3.7
	80–89	132	152	66.5	16.76	**	3	33.3	0.34	ns	1.9
	>90	122	104	59.6	3.87	*	4	50.0	0	ns	3.7
	Total		3358	58.7	107.61	**	248	58.1	8.39	ns	6.9
				Pooled	102.79	**		Pooled	6.48	*	
				Heterogeneity	4.82	ns		Heterogeneity	1.91	ns	

TABLE 5. Infection statistics and distribution of larval *A. simplex* in the tissues of various size classes of Atlantic cod collected in Fortune Bay, Newfoundland, during January 1987. Fillets, napes, liver, and other viscera were examined separately by pepsin–HCl digestion.

Size class (cm)	Number of cod	Prevalence	Abundance $\pm$ SE	Max.	Total <i>A. simplex</i> recovered	Distribution in host tissues (%)			
						Fillets	Napes	Liver	Other viscera
40–44	20	65.0	1.7 $\pm$ 0.45	7	34	5.9	11.8	47.1	35.3
45–49	127	81.1	2.4 $\pm$ 0.22	17	304	3.9	11.8	41.8	42.4
50–54	174	83.3	3.0 $\pm$ 0.24	18	518	4.2	11.6	34.2	50.0
55–59	156	92.3	5.0 $\pm$ 0.40	41	773	2.6	9.6	31.7	56.1
60–64	28	92.9	3.9 $\pm$ 0.62	14	110	4.5	12.7	37.3	45.5
Total					1739	3.5	10.8	34.8	50.8

caution (see McClelland et al. 1983a; Bratney et al. 1990), in general the numbers of *A. simplex* in the flesh of cod in most areas appear to be similar to those observed during the 1950's. Unfortunately, Templeman et al. did not present data on the prevalence of *A. simplex* from their survey, so changes in prevalence cannot be evaluated. However, the data on abundance and density suggest that the numbers of *A. simplex* in the flesh of cod have remained low for stocks off Labrador, the east and northeast coasts of Newfoundland, and the Grand Banks. The reasons for apparent changes in the abundance of *A. simplex* in cod from some areas off the south coast and for the generally higher abundance of larval *A. simplex* in these areas compared with elsewhere remain enigmatic. There is little quantitative information on the numbers of cetacean definitive hosts of *A. simplex* in these areas or on the dietary sources of larval *A. simplex* for cod in Canadian waters (see below).

Comparison of our results with other surveys also indicates that cod from Newfoundland and Labrador generally harbour more *A. simplex* in the musculature than those from other areas off eastern Canada. In previous surveys (McClelland et al. 1983a, 1983b, 1985), counts of nematodes from viscera were also included in calculating the abundance of *A. simplex*; how-

ever, for comparison with present findings, we calculated the abundance in the flesh by using McClelland et al.'s estimates of the proportion of the total *A. simplex* occurring in the fillet and nape of each cod size group. These calculations indicated that the abundance of *A. simplex* was generally ≤ 0.03 in the flesh of cod from the lower Bay of Fundy, Fundian Channel, and eastern Browns Bank and approximately 0.03–0.06 in cod from the Scotian Shelf, with the largest cod (≥ 71 cm) occasionally more heavily infected (0.1–0.2) (from data in McClelland et al. 1983a, 1983b, 1985); a single sample of cod from southern Newfoundland (NAFO division 3O) was more heavily infected (0.27–1.98) and showed infection levels more comparable with those reported here (Table 2). Cod from the west coast of Newfoundland (NAFO divisions 4R–4S) examined by McClelland et al. (1985) appear to have the highest abundances of *A. simplex* in the flesh (0.47–2.91). Surveys have revealed that Atlantic herring from this area are also heavily infected with *A. simplex* (Parsons and Hodder 1971; McGladdery and Burt 1985).

The flesh of Canadian Atlantic cod, however, appears to be lightly infected with *A. simplex* compared with most other stocks in the North Atlantic. Young (1972) reported abun-

dances of 1.01–1.16, 0.39–0.56, and 0.88–3.71 in the flesh of cod collected off the northwest, southwest, and east coasts of Britain during 1968–70, but he apparently did not slice the flesh during examination; therefore, infection levels were probably higher than reported. Also, according to Smith and Wootten (1978), abundances of *A. simplex* in fish from British waters increased dramatically during the 1960's and 1970's. Platt (1975) apparently used techniques similar to those used by Canadian investigators and reported abundances of *A. simplex* in the flesh of cod ranging from 0.7 to 1.7 off Greenland, 1.7 to 2.8 around Iceland, 2.0 to 4.4 around the Faroes, and 4.1 to 10.2 in Arcto-Norwegian cod collected during 1971–73.

Several investigators have provided information on the distribution of *A. simplex* in the tissues of cod (Young 1972; Platt 1975; Wootten and Waddell 1977; Wootten 1978; McClelland et al. 1983a, 1983b, 1985). Present findings are consistent with these surveys which show that (i) the majority of the *A. simplex* in cod occur in the viscera, (ii) most of the *A. simplex* in the flesh reside in the nape, and (iii) there are more nematodes in the flesh on the left side of the fish. McClelland et al. (1985) speculated that the latter finding was due to asymmetry in the gut affecting the route of migration of nematodes into the flesh. Comparison of the results of these surveys also suggests that the distribution of *A. simplex* in the tissues varies among different sizes and stocks of cod, particularly with respect to the proportions occurring in the fillet and nape (see Table 4). However, most of this information is based on fish that were candled or candled and sliced which does not always recover all of the nematodes, so the results may not be directly comparable. Also, the precise location of the cut that divides the flesh into fillet and nape portions has not previously been described but appears to vary between regions according to local preferences (see McClelland et al. 1990). Consequently, as reported here, different findings may partly reflect variation in methodology rather than a real difference in the distribution of the nematodes.

Examination of all tissues of over 500 cod from Fortune Bay (by pepsin–HCl digestion) indicated that ~14.3% of the total *A. simplex* occurred in the flesh. Data presented by McClelland et al. (1985) based on fish of similar size (50–60 cm) and examined by a similar method indicate a somewhat higher proportion (~22%) of the total *A. simplex* burden in the flesh of cod from Nova Scotia; these two data sets are probably more accurate than those obtained by candling and slicing and suggest that there are significant differences in the tissue distribution of *A. simplex* among cod stocks. However, more accurate information from a broader range of sizes and stocks is needed before possible geographic trends in the distribution of *A. simplex* in cod tissues can be discerned. Although it is clearly impractical to use pepsin–HCl digestion on all fish during a large-scale survey, whenever possible, future investigators should examine at least one representative subsample of fish of a wide range of sizes using this or a similar technique (Bratney 1988). Also, to avoid changes in the distribution of nematodes after death of the host, the fish should be deep-frozen immediately after capture (Smith 1984a) and the various tissues digested and examined separately. This would provide more reliable information on the distribution of nematodes among the tissues of fish hosts, particularly for nematodes in the flesh portion which may be of consumer concern.

This study shows that the distribution of larval *A. simplex* in the tissues of cod from Newfoundland and Labrador differs markedly from that of larval *P. decipiens*. Most larvae of *A. simplex* occur in the body cavity and viscera of cod, and of

those inhabiting the flesh the majority occur in the nape rather than the fillet (Table 5; Fig. 2). In contrast, larvae of *P. decipiens* are rare in the body cavity and viscera, and of those occurring in the flesh the majority are found in the fillet portion of small fish but in the nape portion of the largest specimens (Bratney et al. 1990). Both nematodes can occur anywhere in the musculature of cod and they cannot be eliminated by selective trimming of specific portions of the flesh during processing; however, as suggested by Young (1972), removal of the napes, particularly from the larger fish, would remove a substantial proportion of both nematode species.

The increased prevalence and abundance of *A. simplex* with size among cod from Newfoundland and Labrador is consistent with the pattern observed for *A. simplex* among other cod stocks (Young 1972; Platt 1975; Wootten and Waddell 1977; McClelland et al. 1983a, 1983b, 1985) and other fish hosts (McClelland et al. 1990). The larvae of *A. simplex* appear to be long-lived (Smith 1984b) and probably accumulate in fish over time. Small cod harbour relatively few *A. simplex* and possibly acquire the larvae singly from lightly infected invertebrate hosts such as euphausiids (Smith 1983). Crustaceans such as euphausiids, mysids, gammarids, hyperiids, and pandalid shrimps are commonly eaten by cod in waters off Newfoundland and Labrador (Lilly 1987, 1989), but which of these harbour larvae of *A. simplex* remains unknown. Larger cod are mainly piscivorous and probably acquire *A. simplex* in greater numbers from their more heavily infected fish prey. In Newfoundland and Labrador waters, cod commonly feed on capelin, Arctic cod (*Boreogadus saida*), flatfish (Pleuronectidae), and sand lance (*Ammodytes* sp.) (Lilly 1987, 1989). All of these species are potentially important sources of *A. simplex* for cod.

The apparent decline in the prevalence and abundance of *A. simplex* in the musculature of the largest cod in some areas (Table 2) is difficult to explain. Small numbers of necrotic specimens of *A. simplex* were found in the flesh of large cod, but these were too few to suggest that the lower infection level was due to mortality and disintegration of *A. simplex* in the flesh. The decline may be an artifact due to increased difficulty of locating the nematodes in the thicker flesh of the largest cod.

The pepsin–HCl digestion procedure indicated that with the candling and slicing technique used here, less than half of the total numbers of *A. simplex* residing in the flesh were recovered. This result is not surprising because the larvae of *A. simplex* are small, often tightly coiled, and lightly pigmented, rendering them difficult to detect. Data presented by McClelland et al. (1983a) indicate a similar percentage recovery of *A. simplex* from the flesh of cod from Nova Scotia. Consequently, the results obtained during the present survey should be comparable with the results of surveys by McClelland et al. (1983a, 1983b, 1985), although these studies and the present one have underestimated the numbers of *A. simplex* present in the flesh of cod.

There have been several attempts to improve methods for detecting and removing nematodes from fish flesh (Power 1958, 1961; Hafsteinsson et al. 1989). Most of these have been directed towards the larvae of *P. decipiens* which are larger and more unsightly; however, an efficient method of removing *A. simplex* and *P. decipiens* would have obvious benefits from an aesthetic and public health perspective. For accurate scientific surveys, destructive methods such as pepsin–HCl digestion (Stern et al. 1958) or mechanical disintegration (Bratney 1988) are the most efficient in terms of percentage recovery, but they are time-consuming and do not provide information on the precise location of the parasites within the flesh. Can-

dling combined with slicing, or systematic destruction of the flesh (McClelland et al. 1983a, 1983b), remains the standard method of examination during surveys of nematodes in fish flesh.

From a commercial perspective, nondestructive methods are clearly required and examination of fillets on a candling table still remains the only widely used method of detecting nematodes. This method is not particularly efficient (Power 1961; Bratney 1988), but suitable alternatives have yet to be found. An electronic device that uses ultrasound to scan fillets and detect nematodes has shown promise (Hafsteinsson et al. 1989), but has not been developed beyond the experimental stage. More recently, a device that uses lasers to scan fillets has produced excellent results, and a commercial prototype has been constructed and is currently being tested for use in processing plants (J. H. C. Pippy, Department of Fisheries and Oceans, St. John's, Nfld., pers. comm.). However, in the long-term, the development of an automated mechanical device that both detects and removes nematodes may offer the most promising and effective means of ensuring that all nematode parasites are removed from the flesh of fish intended for human consumption.

Although several hundred thousand metric tons of Atlantic cod are caught each year by Canadian fishermen and sold for human consumption, to date there have been no confirmed cases of human infection with *A. simplex* that can be directly attributed to Canadian Atlantic cod. There are several possible reasons for the absence of human infections. Cod are usually cooked before consumption and have often been previously frozen; both of these procedures, when conducted properly (see Bier 1976; Food and Drug Administration 1987; Deardorff and Throm 1988), will readily kill the larvae of *A. simplex*. Also, the flesh of Canadian cod is lightly infected with *A. simplex* relative to other fishes such as Pacific salmon whose flesh can be heavily infected (Myers 1979; Rosset et al. 1982; Deardorff and Throm 1988) and is often eaten raw. Furthermore, Nascetti et al. (1986) and Orecchia et al. (1986) have shown that the genus *Anisakis* in the North Atlantic consists of more than one species, and these findings may be significant from a public health perspective. There may be differences in the geographic distribution, fish hosts, pathogenicity, and thermal tolerance of the different species of *Anisakis* that could be important in understanding the epidemiology of human cases of infection. These aspects of the biology of each species of *Anisakis* should be explored, particularly for those species infecting fishes that are eaten raw and thereby constitute a potential source of infection for humans.

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Numerical Definition of Groundfish Assemblages Caught Off the Coasts of Oregon and Washington Using Commercial Fishing Strategies

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Numerical definition of species caught together by the groundfish trawl fishery operating off the Oregon and Washington coasts during 1985–87 indicated six major assemblages of species. Observers on commercial vessels recorded data allowing estimation of the weights of commercially important species caught in each tow. Assemblages were selected based on consistencies in three types of analysis of the species weights: detrended correspondence ordination, two-way indicator species clustering, and Bray–Curtis group average clustering. Two of the assemblages were dominated by a single species, one consisting largely of smooth pink shrimp (*Pandalus jordani*) and the other primarily of widow rockfish (*Sebastes entomelas*). The other assemblages identified were a deepwater rockfish assemblage, a deepwater Dover sole assemblage, a nearshore mixed-species assemblage, and a bottom rockfish assemblage. These assemblages of commercially cooccurring species may be treated as units in developing mixed-species management plans. The deepwater rockfish assemblage we identify has not been previously described.

La définition numérique des espèces capturées par les chalutiers qui pêchaient le poisson de fond au large des côtes des États de l'Oregon et de Washington au cours de la période 1985–1987 a indiqué six assemblages d'espèces principaux. Des observateurs se trouvant sur des navires commerciaux ont relevé des données permettant l'estimation des poids des espèces commercialement importantes capturées dans chaque trait. Les assemblages ont été choisis en fonction de la conformité dans trois types d'analyse des poids des espèces : ordination de correspondance à tendance temporelle éliminée, groupement des espèces à indicateur double et groupement de moyenne de groupe de Bray–Curtis. Deux des assemblages étaient dominés par une seule espèce, un consistant largement en crevettes océaniques (*Pandalus jordani*) et l'autre principalement en veuves (*Sebastes entomelas*). Les autres assemblages identifiés étaient : un assemblage de sébaste d'eau profonde, un assemblage de sole de Douvres d'eau profonde, un assemblage d'espèces mélangées situé près du rivage et un assemblage de sébaste de fond. Ces assemblages d'espèces présentes commercialement présentes simultanément peuvent être traités comme des unités dans l'élaboration de plans de gestion d'espèces mélangées. L'assemblage de sébaste d'eau profonde que nous avons identifié n'avait pas été décrit précédemment.

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The trawl fishermen operating off the coasts of Oregon and Washington often catch mixed species of groundfish including rockfishes and thornyheads (*Scorpaenidae*), Pacific whiting (Pacific hake) (*Merluccius productus*), flatfishes (*Pleuronectidae* and *Bothidae*), and sablefish (*Anoplopoma fimbria*). Although in the past, these fishes have been managed on an individual species basis, it is now recognized that this can result in excessive waste of incidental catch or overharvest of the least productive species (Paulik et al. 1967; Pikitch 1991). Managers have begun to set trip limits based on species complexes (PFMC 1990), and mixed-species models have been developed to assess the effects of technological interactions (Murawski 1984; Pikitch 1987a). To be effective, these approaches require accurate knowledge of which species are consistently caught together.

Based on qualitative knowledge of the fishery, we would expect five major assemblages of species in the commercial catches. Using information from managers and fishermen, Pikitch et al. (1988) described five major West Coast groundfish strategies which target (intend to catch) different assemblages. Assuming that strategies are accurate and effective, the assemblages caught by the trawl fishery would include (1) a bottom rockfish assemblage (BRF) consisting of rockfish (*Sebastes* spp.), (2) a midwater assemblage (MID), including widow rockfish (*Sebastes entomelas*) and Pacific whiting, (3) a deepwater Dover sole assemblage (DWD), primarily Dover sole (*Microstomus pacificus*), along with sablefish and thornyheads (*Sebastolobus* spp.), (4) a nearshore mixed-species assemblage (NSM) consisting of flatfish, and (5) a shrimp assemblage (SHR), primarily smooth pink shrimp (*Pandalus jordani*).

Quantitative definition of trawl assemblages for the area has been accomplished using research or logbook data, but the resulting assemblages may not accurately represent those caught commercially. Research data analyzed by Alverson (1953),

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Hitz and Alverson (1963), Day and Percy (1968), Percy (1978), Gabriel and Tyler (1980), Percy et al. (1982), and K. L. Weinberg (AFSC NMFS, NOAA, 7600 Sand Point Way N.E., Seattle, WA 98115-0070, unpubl. data) were generally collected using standardized strategies with one or two gears. The commercial fishery uses six gears and probably operates with a variety of strategies (Pikitch et al. 1988). Logbook data, analyzed by Tyler et al. (1984) and Rickey and Lai (1990), do not include discarded fish, have low resolution for rockfish species, and have not been verified for accuracy or consistency. Prior definitions using both types of data are difficult to assess for accuracy, since they generally relied on one or two methods of analysis, which varied between studies.

Quantitative definition using unbiased data and consistencies among a variety of methods of analysis could provide managers and modelers with a more accurate description of the commercially caught assemblages and help assess the previously defined commercial strategies. Data collected by observers on commercial vessels would be relatively accurate and unbiased, assuming that fishermen's behavior on boats that allow observers is representative of commercial fishing behavior. Assemblages determined using a combination of ordination and classification techniques would have greater reliability than definition based on any one method alone (Gauch 1980; Gabriel and Murawski 1985). Consistencies between methods could also allow determination of a few assemblages which reflect major sources of variation in the data, without relying on knowledge of external factors, such as correlation with environmental variables.

The specific objectives of this research were to (1) quantitatively define assemblages of fish caught in the commercial trawl fishery off the coasts of Oregon and Washington using data collected by observers on commercial vessels, (2) assess the accuracy/effectiveness of the strategies described by Pikitch et al. (1988) by comparing tows designated by strategy with tows designated by the defined assemblages and, (3) develop a method of using consistencies in three data analysis techniques to select the assemblages.

Materials and Methods

Data Collection

Observers of normal fishing operations on commercial fishing vessels collected the data (1469 tows) during 1985–87 (Pikitch 1987b). The northern and southern boundaries of the study were 48°42' and 42°60' latitude, respectively, primarily within the INPFC Columbia Management Area. Participation in the study was voluntary and included vessels using bottom, midwater, and shrimp trawls. The skipper and/or observer visually estimated the total weight of the catch from a single tow. The observer then took a random sample from each catch, or examined the entire catch if the total weight was sufficiently small. The weight of each species retained or discarded in the sample was recorded. Total weights of the various species kept or discarded in the catch were estimated by multiplying the sampled weight of the species by the ratio of the total catch weight to the total sample weight. Based on the gear used, depth fished, and species targeted, observers designated a predefined trawling strategy (Pikitch et al. 1988) for each tow. To clarify the distinction between the strategies and the assemblages they are expected to catch, which were described earlier, we added an S in front of the acronyms used by Pikitch et al. (1988) when referring to the strategies. These strategies were (1) bottom rockfish trawling (SBRF): tows conducted

using roller gear on the ocean bottom, with rockfish as the intended catch, (2) midwater trawling (SMID): tows conducted using midwater trawl gear above the bottom, targeting on widow rockfish and Pacific whiting, (3) deepwater Dover trawling (SDWD): bottom tows conducted in areas exceeding 183 m, using mud gear, roller gear, or mud–roller combination gear, with targeting primarily on Dover sole, along with sablefish and thornyheads, (4) nearshore mixed-species trawling (SNSM): tows conducted using mud gear on the bottom in less than 183 m with flatfish as primary targets, and (5) shrimp trawling (SSHR): tows conducted using shrimp gear, targeting on pink shrimp.

Data Preparation

We used a data base consisting of total species weights in each catch and corrected and reduced the data base by eliminating certain tows and species. Ranges, plots, and charts of the data were examined and outliers that were obvious errors were removed. To fit the available clustering capacity (amount of computer memory) and make the results more interpretable, we used only those species deemed commercially important in defining assemblages. The species selected were those that the fishermen identified as target species or those species that composed at least 1% of the estimated total of all catches sampled. We eliminated tows without any catch, lacking a sample, or missing information on the weight of a species. Tows with missing weights of Pacific halibut (*Hippoglossus stenolepis*) or Pacific salmon (*Oncorhynchus* spp.) were not eliminated. Observers usually did not weigh those two species, as it was illegal to retain them onboard.

Description of Analyses

We analyzed the data using an ordination technique and two opposite types of hierarchical classification techniques. Ordination was used to represent catch and species relationships in a low-dimensional space (Gauch 1980). Hierarchical classification was used to place catches into groups, with relationships among groups demonstrated by a dendrogram (Gauch 1980). We employed two types of hierarchical classification: agglomerative, which starts with individual hauls and progressively combines them, and divisive, which starts with all the hauls and progressively divides them.

For ordination, we selected detrended correspondence analysis (DCA) (Hill 1979a), which is a modification of reciprocal averaging and iteratively maximizes the correspondence between the species and catch ordinations. DCA derives a series of ordination axes. Each axis consists of a set of species scores and a corresponding set of catch scores, which are weighted averages of the species scores (Hill and Gauch 1980). Each axis has an eigenvalue which represents the amount of correspondence between species and catch scores on that axis. The axes are scaled so that on a species axis, a species may be expected to appear, rise to its mode of abundance, and disappear in about 4 units, and on a catch axis, a full turnover in species composition occurs over 4 units (Hill and Gauch 1980). DCA is preferable to other ordination techniques in that it does not require a linear relationship between species and catches, eliminates any systematic relationship between the series of ordination axes, and scales the axes so that the dispersion of species scores within samples is constant.

The hierarchical agglomerative technique used to classify the hauls was based on the Bray–Curtis dissimilarity index (Bray and Curtis 1957) with group average fusion criteria (Sneath and

Sokal 1973).³ The Bray–Curtis index has been used extensively in marine ecology (Boesch 1977) and tends to be good at reflecting abiotic aspects (Clifford and Stephenson 1975). Group average fusion is the most widely used clustering method in aquatic ecology and introduces relatively little distortion to the relationships expressed in the matrix (Boesch 1977).

The hierarchical divisive clustering technique used was two-way indicator species analysis (TWINSPAN) (Hill 1979b). TWINSPAN was selected because it uses information on all the species (it is polythetic rather than monothetic), provides an objective method of splitting ordinations, and has minimal computer space and time requirements (Gauch 1980). TWINSPAN operates by dividing ordinations in half. It constructs three ordinations: a “primary” ordination using reciprocal averaging, a “refined” ordination using as a basis the species preferential to one side or the other of the primary ordination, and an “indicator” ordination based on only the most highly preferential species. The refined ordination generally determines the division, while the indicator ordination describes it. To account for differences in abundance, we designated cutoff values so that each species could be treated as four separate “species”, based on abundance in the haul. Since cutoff values had to be the same for all species, we computed the means of target species abundances and designated cutoff values as absence of catch, the minimum of the target species means, the mean of target species means, and the maximum mean.

Assemblage Determination

To look for consistent assemblage patterns in the three methods of data analysis, we first determined the maximum number of clusters to consider for each clustering method. We utilized dendrograms, illustrating the way groups hierarchically combined or divided, with the number of clusters increasing with decreasing levels on the dendrograms. We began by selecting a level of agglomeration or division which resulted in two groups of catches and used those cluster designations on plots of the DCA catch scores. We plotted the DCA catch scores for two axes at a time (x and y), with each catch designated by cluster. This was done for both the Bray–Curtis and TWINSPAN cluster designations. The levels were then changed to increase the number of groups until the cluster designated for catches with scores near one end of each DCA axis was different than the cluster designated for the catches with scores near the other end of the axis. If the groups did not separate similarly to the axis scores at any level of clustering, the axis was not used. At each level of clustering, we only considered catch groups that contained more than 1% of the catches. Clusters with less than 1% of the catches were not split off in the TWINSPAN clustering and were eliminated from the Bray–Curtis clustering.

After the maximum number of clusters to consider was determined, clusters were combined or recombined to higher levels on the dendrograms to achieve consistency in catch placement on the DCA axes between the two methods of clustering. This was done given that different clusters were still associated with opposite extremes of the selected DCA axes. For instance, at the minimum levels on the dendrograms, the Bray–Curtis clustering might have determined one cluster for a given area on a DCA axes plot and TWINSPAN two clusters in the same area. If those two clusters recombined to one cluster at a higher level

on the TWINSPAN dendrogram, that level was selected. If consistency could not be achieved while maintaining different cluster designations for catches with scores near opposite ends of the axes, a cluster was considered an inconsistent assemblage.

We then compared the species associated with the selected DCA axes and the selected catch clusters for each clustering method. The species associations with the catch clusters were emphasized rather than species clusters themselves, since we desired species to be allowed to associate with more than one group. Plots of DCA species scores on the selected DCA axes were examined and species associated with the clusters were outlined on the plots. For the Bray–Curtis catch clusters, we examined two measures of species association. One measure expressed which species were caught in the greatest abundance (percentage of total weight in the cluster). The second measure indicated additional species which, although in low abundance in all clusters, were caught selectively in certain clusters (average weight in a cluster divided by the average weight caught in all the clusters) (Boesch 1977). For TWINSPAN, we looked at the indicator species for each cluster.

The defined assemblages were then assigned names based on their similarity to assemblages expected from the strategy definitions. A table was derived showing the number of tows for each combination of strategy and assemblage designation. An assemblage was given the name and acronym of the expected assemblage if most of the tows placed in that assemblage were designated as that strategy and the species associated with the assemblage were similar to the species expected given that strategy. If there was not a strong agreement between an assemblage and any one strategy, the assemblage was given a name based on the species associated with it.

Results

Preliminary Analysis

The species abundance data matrix on which we based the analyses contained information on 1351 of the 1469 tows and 26 of the 178 species found in the catches. Five species were not identified as target species, but had total catches greater than 15 323 kg, which was 1% of all the weight sampled. Those species were longnose skate (*Raja rhina*), spiny dogfish (*Squalus acanthias*), Pacific whiting, sharpchin rockfish (*Sebastes zacentrus*), and yellowmouth rockfish (*Sebastes reedi*). Six species were identified as targets by the fishermen, but did not constitute at least 1% of the total catches sampled: sanddab (*Citharichthys* spp.), starry flounder (*Platichthys stellatus*), sand sole (*Psettichthys melanostictus*), Pacific cod (*Gadus macrocephalus*), bocaccio (*Sebastes paucispinis*), and yelloweye rockfish (*Sebastes ruberrimus*). The remaining 15 species were targeted and constituted >1% of the catch. These comprised arrowtooth flounder (*Atheresthes stomias*), petrale sole (*Eopsetta jordani*), English sole (*Pleuronectes vetulus*, previously *Parophrys vetulus*), Dover sole, rex sole (*Errex zachirus*, previously *Glyptocephalus zachirus*), sablefish, lingcod (*Ophiodon elongatus*), shortspine thornyhead (*Sebastolobus alascanus*), Pacific ocean perch (*Sebastes alutus*), darkblotched rockfish (*Sebastes crameri*), splitnose rockfish (*Sebastes diploproa*), widow rockfish, yellowtail rockfish (*Sebastes flavidus*), canary rockfish (*Sebastes pinniger*), and smooth pink shrimp.

We eliminated a total of 118 tows from the species abundance matrix: three tows had uncorrectable errors, there was no sample in 29 tows and no catch in 59 tows, and 27 tows had missing

³A FORTRAN program to form the matrix and a SAS Institute, Inc. (1988) program to compute the group averages are available upon request.

information on the weight of a nonprohibited species. We included an additional 390 tows with missing weights for Pacific salmon and Pacific halibut. These catches may have slightly overestimated weights for the selected species because the catch weights included the prohibited species but the sample weights did not.

Assemblage Definition

We derived six consistent assemblages based on the three methods of analysis (Fig. 1 and 2). Two of the assemblages were dominated by single species, SHR and the widow rockfish assemblage (WID) (Fig. 2). The NSM assemblage contained sanddab, English sole, sand sole, starry flounder, and petrale sole (Fig. 2). The BRF assemblage contained yellowtail rockfish, canary rockfish, yelloweye rockfish, lingcod, bocaccio, and sharpchin rockfish. The DWD assemblage was primarily Dover sole and sablefish. The deepwater rockfish assemblage (DWR) contained darkblotched rockfish, Pacific ocean perch, splitnose rockfish, yellowmouth rockfish, and sharpchin rockfish. Other species considered were associated with the assemblages, but to a lesser degree (Fig. 2).

We found strong agreement between many of the strategy designations and the assemblage designations, but there were also some differences (Table 1). The tows placed in the SHR, NSM, and BFR assemblages were almost entirely designated

the respectively named strategies, and the species associated with the assemblages were similar to the targeted species of those strategies. The DWD tows were mainly designated as SDWD, with associated species similar to the DWD targeted species, but also had a number of SNSM designations (Table 1). Two of the consistent assemblages were not in strong agreement with a strategy. WID was associated with widow rockfish and contained most of the tows designated SMID, but also had a substantial number of SBRF tows. DWR was associated with rockfish species found in relatively deep water and contained tows designated as mainly SBRF or SDWD.

The six consistent assemblages designated were the result of 82% agreement between the Bray-Curtis clusters and the TWINSpan clusters (Table 1) and had varying degrees of overlap on the DCA axes (Fig. 1 and 2). The DCA program derived four axes in order of decreasing correspondence between the catch and species scores (Table 2). The first axis appeared to represent a general separation of catches containing rockfish species from those containing flatfish species, but also separated the rockfish catches into the WID, BRF, and DWR assemblages (Fig. 1 and 2). The second axis separated SHR from the other assemblages, particularly from DWR, NSM, and DWD. The third axis served to separate NSM from DWR and, to a lesser extent, DWD from NSM and DWR. The fourth axis was not used, since it represented a separation of rockfish

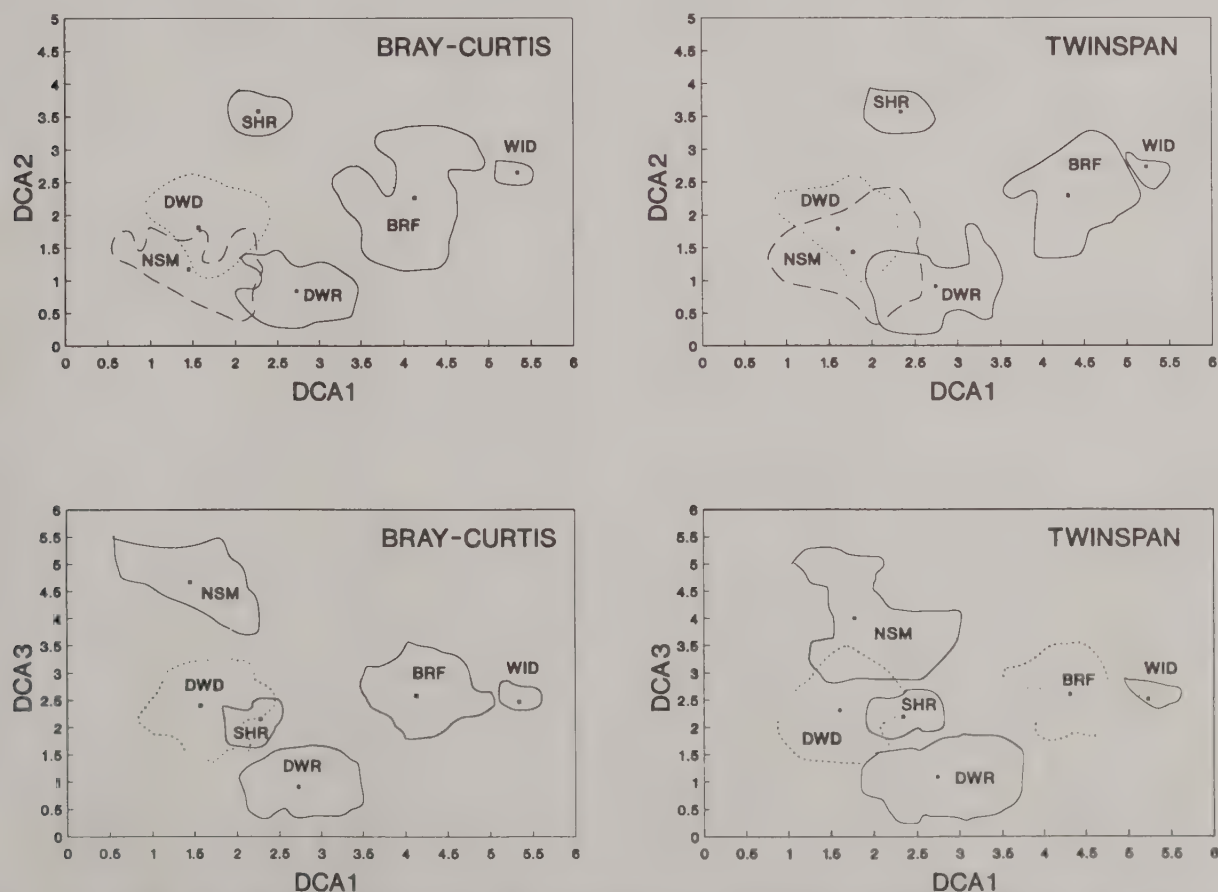


FIG. 1. Consistency of catch separations as demonstrated by the concentration of DCA catch scores for the Bray-Curtis clusters and TWINSpan clusters. The mean catch scores for the clusters are designated by a dot, while the outlines enclose 75% of the hauls in the cluster closest to the mean. The means are labeled to show the assemblage names assigned the respective clusters.

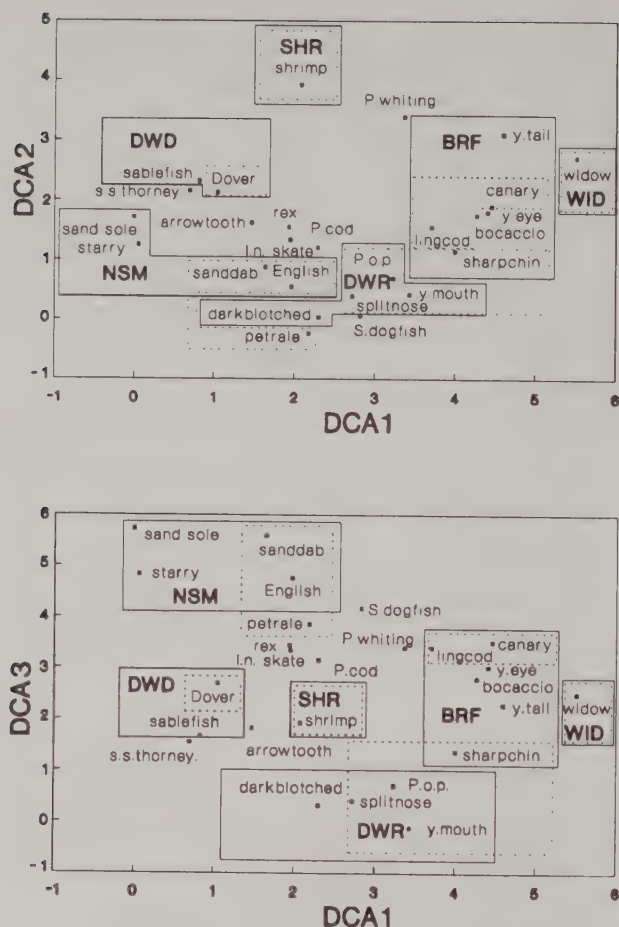


FIG. 2. Consistency in species associated with the catch clusters labeled by assemblage name. Species scores are designated by dots and labeled with species names. Dotted boxes enclose the indicator species of the TWINSpan clusters and solid boxes enclose the species associated with the Bray-Curtis clusters. Species selected for Bray-Curtis were those averaging >20% by weight in the catches and/or >4 times the average weight.

species inconsistent with the TWINSpan separation of species. The Bray-Curtis clustering tended to provide better separations on the DCA axes, particularly in separating DWD from NSM (Fig. 1).

Nine Bray-Curtis clusters were required to derive different groups at opposite ends of DCA axes 1–3 (Fig. 3). To achieve consistency with the clustering species associations, three Bray-Curtis clusters were combined to form BRF (Fig. 3). These clusters were associated with yellowtail rockfish, canary rockfish, or sharpchin rockfish. There was one inconsistent assemblage, named DOG because it was associated with spiny dogfish. It was primarily part of the TWINSpan DWD, but could not be included in the Bray-Curtis DWD without first combining DWR with DWD, and DWR was associated with an extreme of DCA axis 3 (Fig. 1 and 2). The species associations with the consistent Bray-Curtis assemblages, which were the basis of the outlines in Fig. 2, are shown in Table 3.

TWINSpan showed different clusters at opposite ends of the first three DCA axes after four divisions, resulting in 15 groups (Fig. 4). WID was not divided in the first division, since it included less than 1% of the catches. Many of the clusters

were recombined to be consistent with the other methods of analysis (Fig. 4). One of the four clusters combined to form NSM could be considered a transition cluster. It was intermediate in the separation of NSM and DWD on DCA axis 3, was placed primarily in the Bray-Curtis DWD, and was associated with sablefish, arrowtooth flounder, and Dover sole, along with petrale sole and sanddab. The species associations with the consistent TWINSpan assemblages, which were outlined in Fig. 2, are shown in detail in Table 4.

Discussion

Our results suggest that widow rockfish and smooth pink shrimp may be managed as separate species, but the other species could be managed as part of assemblages. As of October 1990, trip limit restrictions were in effect for two multispecies assemblages: a *Sebastes* complex (all rockfish except widow rockfish, Pacific ocean perch, thornyheads, and shortbelly rockfish (*Sebastes jordani*)) and a deepwater complex, including sablefish, Dover sole, and thornyheads (PFMC 1990). Sablefish was additionally limited to 25% of the deepwater complex, yellowtail to 20–30% of the *Sebastes* complex, and Pacific ocean perch to 20% of all fish onboard within a given range of weights. Our findings agreed with use of the deepwater complex; Dover sole and sablefish were highly associated with our DWD assemblage, and thornyheads had the next closest association (Fig. 2; Table 3). In addition, sablefish averaged close to 25% by weight in the DWD catches (Table 3). We determined that the *Sebastes* complex could be divided into two assemblages, DWR and BRF, with Pacific ocean perch approximately 20% of DWR and yellowtail rockfish about 50% of BRF (Table 3). The possibility of setting separate trip limits for BRF and DWR could allow managers more flexibility in managing rockfish in the future.

To be useful to managers and modelers, the assemblages we defined should be persistent over time. The assemblages could change if the species mixes available to the fishermen change or the fishermen change the strategies they use to catch the species. The species available could change as a result of environmental changes or harvesting pressures. Strategies employed may change based on market prices, regulations, or new technology.

Although monitoring the fishery over time using our same methodology would be desirable to examine persistence of the assemblages, comparison of our study with other trawl studies conducted in the same area does indicate some persistence which is independent of methodology. Assemblages were defined previously with various methods of data analysis, using data collected with a variety of strategies, from time periods before, during, and after our data base. In spite of this, some consistencies were evident between our assemblages and those defined by other authors. A species association similar to our NSM was designated by Alverson (1953), Day and Percy (1968), Percy (1978), Gabriel and Tyler (1980), and Tyler et al. (1984). Prior studies determined a deepwater assemblage, although the DWR and DWD assemblages were often combined and a separate DWR was never distinguished. Alverson (1953), Hitz and Alverson (1963), Gabriel and Tyler (1980), and K. L. Weinberg (unpubl. data) defined species associations and assemblages using a combination of DWD and DWR species. Percy et al. (1982) determined a sablefish, Dover sole, and thornyhead cluster when investigating deepwater areas. Rickey and Lai (1990) analyzed only four species in defining a deepwater complex, but determined that Dover sole and

TABLE 1. Number of tows in strategies designated by observers and in assemblages determined by Bray-Curtis catch clusters, TWINSpan catch clusters, and both, designated as catching the same assemblage by both methods of clustering (tows that were inconsistent were not included).

	Designated strategy					Total
	SNSM	SDWD	SSHR	SBRF	SMID	
Bray-Curtis						
NSM	101	1	0	5	0	107
DWD	148	439	0	29	2	618
DWR	0	33	2	77	0	112
SHR	0	0	217	0	0	217
BRF	4	4	2	172	2	184
WID	0	0	0	25	24	49
DOG	8	13	1	17	0	39
TWINSpan						
NSM	192	4	6	8	0	210
DWD	73	455	3	44	0	575
DWR	0	33	1	98	0	132
SHR	0	0	211	4	7	222
BRF	1	0	2	162	2	167
WID	0	0	0	19	26	45
Both						
NSM	100	1	0	4	0	105
DWD	63	428	1	17	0	509
DWR	0	25	0	72	0	97
SHR	0	0	209	0	0	209
BRF	0	0	1	140	2	143
WID	0	0	0	15	24	39

TABLE 2. DCA species scores on axes 1-4, where scores are 100 times the units and closely related species have similar scores. Eigenvalues (EIG) represent the amount of correspondence between the species scores and the sample scores for that axis. DCA species axes are plotted in Fig. 2.

DCA1 (EIG = 0.901)		DCA2 (EIG = 0.718)		DCA3 (EIG = 0.525)		DCA4 (EIG = 0.358)	
Widow rockfish	551	Smooth pink shrimp	392	Sand sole	572	Canary rockfish	344
Yellowtail rockfish	460	Pacific whiting	339	Sanddab	559	Bocaccio	314
Canary rockfish	446	Yellowtail rockfish	310	Starry flounder	483	Yelloweye rockfish	306
Yelloweye rockfish	441	Widow rockfish	271	English sole	475	Darkblotched rockfish	277
Bocaccio	427	Sablefish	232	Spiny dogfish	417	Smooth pink shrimp	273
Sharpchin rockfish	400	Shortspine thornyhead	215	Petrale sole	385	Sand sole	272
Lingcod	370	Dover sole	212	Canary rockfish	351	Splitnose rockfish	265
Yellowmouth rockfish	343	Canary rockfish	190	Rex sole	344	Starry flounder	261
Pacific whiting	336	Yelloweye rockfish	180	Pacific whiting	340	Pacific ocean perch	232
Pacific ocean perch	323	Bocaccio	175	Lingcod	340	Yellowmouth rockfish	225
Spiny dogfish	282	Sand sole	171	Longnose skate	334	Sablefish	197
Splitnose rockfish	272	Arrowtooth flounder	162	Pacific cod	315	Shortspine thornyhead	187
Darkblotched rockfish	230	Lingcod	155	Yelloweye rockfish	302	Arrowtooth flounder	180
Pacific cod	229	Rex sole	155	Bocaccio	280	Widow rockfish	161
Petrale sole	218	Longnose skate	134	Dover sole	270	Lingcod	147
Smooth pink shrimp	207	Starry flounder	125	Widow rockfish	249	Sanddab	135
English sole	196	Pacific cod	121	Yellowtail rockfish	228	English sole	131
Longnose skate	195	Sharpchin rockfish	115	Smooth pink shrimp	192	Pacific whiting	91
Rex sole	193	Sanddab	88	Arrowtooth flounder	182	Dover sole	91
Sanddab	164	Pacific ocean perch	70	Sablefish	166	Spiny dogfish	78
Arrowtooth flounder	147	English sole	55	Shortspine thornyhead	154	Longnose skate	73
Dover sole	104	Yellowmouth rockfish	43	Sharpchin rockfish	137	Rex sole	59
Sablefish	81	Splitnose rockfish	40	Pacific ocean perch	70	Petrale sole	59
Shortspine thornyhead	70	Spiny dogfish	7	Splitnose rockfish	40	Pacific cod	54
Starry flounder	5	Darkblotched rockfish	4	Darkblotched rockfish	31	Sharpchin rockfish	25
Sand sole	1	Petrale sole	23	Yellowmouth rockfish	-12	Yellowtail rockfish	0

sablefish had the strongest association, followed by thornyheads and then arrowtooth flounder. One study defined an assemblage similar to BRF. K. L. Weinberg (unpubl. data) analyzed Scorpaenidae only and described an assemblage which

was associated with yellowtail and canary rockfishes.

Differences that did exist between the assemblages we defined and those defined previously were primarily a result of different placement of the boundaries separating the assem-

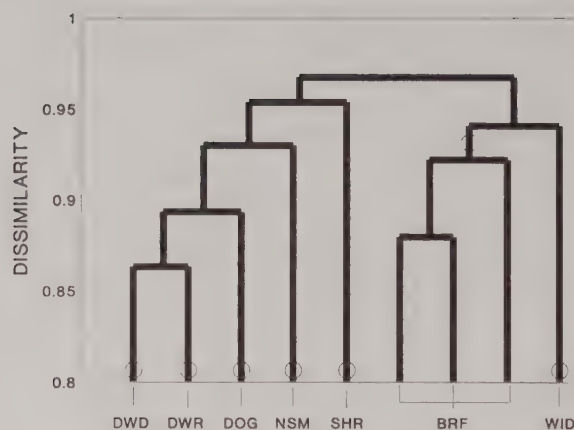


FIG. 3. Dendrogram of Bray-Curtis cluster combinations at increasing levels of dissimilarity. Level of dissimilarity selected in determining labeled assemblages is indicated by a circle.

blages. Not only were DWD and DWR often combined, but some studies determined additional assemblages which could be considered intermediate between our assemblages. An assemblage intermediate between NSM and DWD was described by Hitz and Alverson (1963), Day and Percy (1968), Percy (1978), and Tyler et al. (1984), between DWR and BRF by Gabriel and Tyler (1980), and between BRF and WID by K. L. Weinberg (unpubl. data). The one major difference

between our assemblages and those defined earlier which could not be attributed to differences in boundary placement was Gabriel and Tyler's (1980) definition of an assemblage which included Dover sole and Pacific whiting with canary rockfish. We did not find that those three species had any close association.

Differences among the assemblage definitions could result from many factors. It is possible that the location of the boundaries between assemblages depended on the method of data analysis employed. The previous studies generally used one or two types of analysis and stressed selection of assemblages with hauls made in the same depth range or area. For instance, we could have selected levels in the clustering that combined DWR and DWD, and may have done so if we had not considered the DCA axes separation of the two assemblages. Differences could also result from variations in the data. As stated previously, logbook and research data may not accurately reflect the commercial catch. The targeting and strategies used by commercial fishermen may have resulted in distinctions between assemblages which were not present in research catches. The research cruises were conducted using more restrictive time periods, bottom depths, and gear types than used by the commercial fishery. The studies that combined DWR and DWD were all based on research cruise data. The inclusion of discarded fish in the observer data versus the logbook data could also have affected the boundaries between assemblages. It is also possible that observer coverage of the commercial fleet was not representative of the total commercial effort. Another factor that may have caused the differences could be changes in the relative

TABLE 3. Species associations with Bray-Curtis catch clusters designated by assemblage name. Two indices of species associations are shown: %, average percent by weight in the hauls; $\bar{x}$ ratio of average weight in the assemblage catches to average weight caught for that species overall (where 4 = greater than or equal to 4 times the average, 3 = 2-4 times the average, 2 = average to 2 times, 1 = 0 to below average, and 0 = none).

Species	Assemblage													
	DWD		DWR		DOG		NSM		SHR		BRF		WID	
	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$
Dover sole	27	2	3	1	7	1	2	1	<1	1	<1	1	<1	1
Sablefish	21	2	5	1	3	1	<1	1	1	1	<1	1	<1	1
Shortspine thornyhead	7	3	2	1	1	1	0	0	<1	1	<1	1	<1	1
Arrowtooth flounder	11	2	2	1	5	2	<1	1	1	1	<1	1	0	0
Pacific ocean perch	3	1	21	4	1	1	<1	1	<1	1	1	1	<1	2
Darkblotched rockfish	3	1	31	4	2	1	0	0	<1	1	<1	1	<1	1
Yellowmouth rockfish	<1	1	14	4	<1	1	0	0	<1	1	1	1	<1	1
Splitnose rockfish	1	1	9	4	<1	2	0	0	<1	1	<1	1	0	0
Spiny dogfish	2	1	<1	1	62	4	<1	1	<1	1	<1	1	<1	1
Petrale sole	5	2	<1	1	4	3	10	2	<1	1	<1	1	<1	1
Sanddab	<1	0	0	0	<1	1	25	4	<1	1	<1	1	0	0
English sole	1	2	<1	1	<1	2	22	4	<1	1	<1	1	0	0
Sand sole	<1	1	0	0	0	0	18	4	0	0	0	0	0	0
Starry flounder	<1	1	0	0	0	0	2	4	0	0	0	0	0	0
Longnose skate	3	2	<1	1	1	2	6	2	<1	1	<1	1	<1	1
Rex sole	4	2	<1	1	2	2	6	2	1	1	<1	1	<1	1
Pacific whiting	7	1	3	1	2	1	6	1	12	1	1	1	2	1
Smooth pink shrimp	<1	1	0	0	0	0	0	0	78	4	<1	1	0	0
Pacific cod	1	2	<1	1	<1	1	<1	1	<1	1	<1	1	<1	1
Lingcod	1	1	<1	1	2	3	1	1	<1	1	7	4	<1	1
Yellowtail rockfish	<1	1	<1	1	2	1	<1	1	2	1	49	4	2	2
Canary rockfish	<1	1	<1	1	<1	1	<1	1	<1	1	18	4	1	3
Sharpchin rockfish	<1	1	4	3	2	3	<1	1	<1	1	9	4	<1	2
Bocaccio	<1	1	<1	1	<1	1	<1	1	<1	1	3	4	<1	3
Yelloweye rockfish	<1	1	<1	1	<1	1	<1	1	0	0	2	4	<1	2
Widow rockfish	<1	1	3	1	<1	1	0	0	<1	1	1	1	92	4

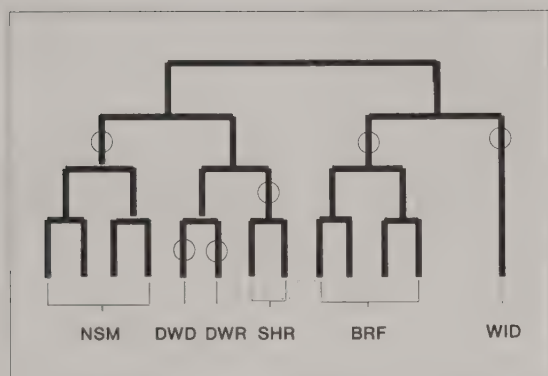


FIG. 4. Dendrogram of TWINSpan haul cluster divisions at progressive separations. Level of separation selected in determining labeled assemblages is indicated by a circle.

amounts of the species within assemblages over time. Most of the other studies used data collected prior to our data base. Further study is needed to determine the actual reasons for dissimilarities noted between our assemblage definitions and those defined previously.

Some of the strategies defined by Pikitch et al. (1988) based on gear, water depth, and targeted species appeared accurate and effective, allowing prediction of the assemblages caught, but some modifications appeared necessary (Table 1). Comparison of strategy and assemblage designations indicated that the use of shrimp gear was adequate to predict the catch of SHR, and fishermen using midwater gear nearly always caught WID. NSM was caught with mud gear in less than 183 m, but some DWD was also caught in the shallower water. Mud gear used in water greater than 183 m caught DWR as well as DWD. BRF was caught primarily with roller gear, but that gear was also used to catch WID and DWR. The strategies need modification because either targeting was ineffective or samplers, constrained to the five defined strategies, designated strategies based more on the gear and depth than on targeted species. The similarities that were present between the designated strategies and the assemblages imply that targeting was effective on certain assemblages, particularly SHR and WID, and that the strategies generally remained stable over time.

Comparing the results of the three methods of analysis not only led to assemblages relatively independent of method used,

but also allowed us to determine the hierarchical levels to select in the clusterings. A common problem in using hierarchical clustering is determining the level(s) on the dendrogram at which to select the clusters (Boesch 1977). The DCA ordination axes pointed out the major sources of variation in the data which should be included in the selected clusters. By combining or dividing clusters until the groups included the DCA axis separations and were consistent between the two types of clustering, we utilized the strengths in the different clustering methods. Consistent groups formed by the two clustering methods balanced the disadvantages and advantages of each method. TWINSpan started with the data base as a whole and therefore used the maximum amount of information in determining the major breaks in the data, making it a more robust technique than group average fusion (Gauch and Whittaker 1981). It also used the original data rather than a secondary dissimilarity matrix and integrated the classifications of both catches and species (Gauch 1980). A disadvantage is that it was biased towards forming subdivisions of nearly equal size during each division (Boesch 1977). It also had the disadvantage that each split was not treated totally hierarchically, since each cluster split at each level of the dendrogram. Further analysis to determine average ordination distances between clusters would have been required to achieve a fully hierarchical dendrogram (Gauch and Whittaker 1981). Defining species based on abundance levels in TWINSpan allowed consideration of both scarce and dominant species, but had the disadvantage that different abundances of a species were treated as separate species, with arbitrary cutoff values. The Bray-Curtis index with group average fusion clustering utilized the range of abundances of the species, but placed emphasis on dominant species and large hauls (Clifford and Stephenson 1975). Comparing the clusters formed by the two different methods allowed us to better determine the hierarchy of the TWINSpan splittings, and balanced the influence of dominant and scarce species.

Conclusions and Recommendations

Six major assemblages of species could be used by managers and modelers to develop management plans for the trawl fishery operating off the Oregon and Washington coasts. Two of the assemblages were dominated by single species, widow rockfish or smooth pink shrimp. The other four included a deepwater Dover assemblage, a deepwater rockfish assemblage, a bottom rockfish assemblage, and a nearshore mixed-species assem-

TABLE 4. Species associations with TWINSpan haul clusters designated by assemblage name. Species associations shown are the inclusive weight categories (kilograms per haul) of the indicator species as determined by TWINSpan.

Species	Assemblage					
	DWD	DWR	NSM	SHR	BRF	WID
Dover sole	15-933					
Pacific ocean perch		15-126				
Yellowmouth rockfish		>0-126				
Splitnose rockfish		>0-15				
Sharpchin rockfish		>0-15				
Petrale sole			>0-15			
Sanddab			15-126			
English sole			>0-126			
Smooth pink shrimp				15-126		
Lingcod					>0-15	
Canary rockfish					>0-126	
Widow rockfish						15

blage. The separation of the deepwater rockfish assemblage from the bottom rockfish assemblage has not been previously used in setting trip limits, and the separation of a deepwater rockfish assemblage from deepwater Dover was not previously designed quantitatively.

Although we could not unambiguously determine year-to-year persistence of the assemblages based on the limited number of years in our data base, comparison with past studies did indicate that there may be some persistence. There were overall similarities between our findings and assemblages defined previously based on logbook or research data collected in other periods. It is possible that the assemblages caught were based on relatively stable biological associations of the species, which limited the possible technological interactions.

Our results indicated that the strategies defined by Pikitch et al. (1988) need some modification to accurately predict the assemblages caught. These modifications may include designating a separate deepwater rockfish strategy, a widow strategy using roller gear as well as midwater gear, and reducing the depth criteria separating the nearshore mixed-species and deepwater Dover sole strategies.

The method we used to determine the assemblages using consistencies from three methods of analysis could be applied to other data bases. An advantage of our method is that it allows relatively objective determination of assemblages based solely on species abundances in samples. Our method allows definition of groups which reflect the major sources of variation in the data, without requiring knowledge of the factors that cause the variation.

Future studies could include further assessment of the similarities and differences between logbook, research, and observer data. We found general consistencies in assemblages defined from the different types of data, but there was variation in the placement of the boundaries between assemblages. Differences may be more evident or conclusive if comparisons are based on data collected in the same years and analyzed using the same methodology.

Comparison of targeted species with species caught and assessment of the species discarded could be useful. It would help determine if the modifications in the strategies described by Pikitch et al. (1988) are required because targeting was ineffective or because the strategies did not accurately define the behavior of the fishermen during the study.

It would be valuable to define strategies quantitatively for each of the assemblages, based solely on the operational decisions the fishermen make, and without relying on knowledge of the targeted species. It could provide necessary modifications to the strategies as well as aid managers and fishermen in determining how to control the catch of the six assemblages defined in this paper.

Acknowledgements

This project was funded by Captain R. Barry Fisher and NOAA Office of Sea Grant, Department of Commerce, under grant No. NA85AA-D-SG095R/ES-7, the National Marine Fisheries Service (contract NA-85-ABH-00025), the Oregon Department of Fish and Wildlife, and the commercial fishing industry. We are grateful to the fishermen who allowed observers onboard and to the observers who collected and input the data. David McIntire, Bill Hopper, Bryan Coleman, and John Chapman provided programs for clustering and DCA or the computer resources necessary to run them. David McIntire provided instruction regarding the TWINSpan and DCA programs. Jose A. Perez Comas, Susan Hanna, Dan Erickson, and William Lenarz

provided helpful comments on the paper. John Wallace assisted in producing the figures.

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ANNOUNCEMENTS/AVIS

International Congress on Modelling and Simulation

University of Western Australia
6-10 December 1993

Theme: *Modelling change in environmental and socioeconomic systems*

The Congress incorporates the 10th Biennial Conference of the Modelling and Simulation Society of Australia, Inc. (MSSA Inc. (formerly SSA Inc.) is an affiliate of the International Association for Mathematics and Computers in Simulation (IMACS).

MSSA Inc. is an interdisciplinary society which aims to promote, develop, and assist in the study and practice of all areas of modelling and simulation in Australia.

There is an emphasis on applied problems. Many of its members work on environmental, agricultural, and socioeconomic methods and applications. Hence, it is natural that the Congress be a joint meeting with the International Society for Ecological Modelling (ISEM) and the International Environmetrics Society (TIES), as well as with IMACS.

There will be four Presidential and five Keynote addresses. These and many of the parallel sessions of contributed papers will focus on areas of common interest to the international societies and participating Australian societies.

Abstracts are due **8 March 1993**. Full papers are due **2 August 1993**. For further information and registration forms, contact Tony Jakeman, CRES, Institute of Advanced Studies, Australian National University, Canberra ACT 2601, Australia. Telephone: 61 6 249 4742; Fax: 249 0757; Email: tony@cres.anu.edu.au.

Final Announcement The Group for Aquatic Primary Productivity (GAP) 6th International Workshop

7-15 June 1993

National Hydrology Research Institute
Environment Canada, Saskatoon, Saskatchewan, Canada

Theme

Effects of physical forcing on primary production processes in inland and marine environments.

Based on a program of active experimentation and data analysis, the main objectives of the Workshop are to assess the states of knowledge on the Workshop theme, perform joint field experiments using different techniques to test their comparability and reliability, and define major gaps and urgent research needs.

Symposium international sur la modélisation et la simulation

University of Western Australia
6 au 10 décembre 1993

Thème : *Changement de modélisation dans les systèmes environnementaux et socio-économiques*

Le symposium s'associe à la 10^e conférence biennale de la Modelling and Simulation Society of Australia Inc. (MSSA Inc.) (appelée auparavant SSA Inc.) laquelle est membre de l'International Association for Mathematics and Computers in Simulation (IMACS).

La MSSA Inc., société interdisciplinaire, veut jouer un rôle actif dans l'intensification de l'étude et de la mise en application de tous les aspects de la modélisation et de la simulation en Australie.

La MSSA met l'accent sur les difficultés d'application. Nombre de ses membres travaillent dans les domaines de l'environnement, de l'agriculture et de la socio-économique. Il est donc tout à fait normal que le symposium se tienne en collaboration avec l'International Society for Ecological Modelling (ISEM) et the International Environmetrics Society (TIES).

Le symposium comportera quatre discours présidentiels et cinq discours-programme. Ces discours, et nombre des séances parallèles de présentation de documents, porteront notamment sur des domaines d'intérêt commun pour les sociétés internationales et australiennes participantes.

Les échéances de présentation sont les suivantes : résumés, **8 mars 1993**; documents entiers, **2 août 1993**. Pour de plus amples renseignements ou pour obtenir des formulaires d'inscription, communiquer avec Tony Jakeman, CRES, Institute of Advanced Studies, Australian National University, Canberra ACT 2601, Australie. Téléphone : 61 6 249 4742; télécopieur : 249 0757; courrier électronique : tony@cres.anu.edu.au.

Le Groupe de productivité primaire aquatique (GPA) 6^e atelier international

7-15 juin 1993

Institut national de recherche en hydrologie
Environnement Canada, Saskatoon (Saskatchewan), Canada

Thème

Effets du forçage physique sur les processus de production primaire en milieux terrestre et marin.

S'inspirant d'un programme d'expérimentation active et d'analyse de données, les principaux objectifs de l'atelier sont évaluer les états des connaissances sur le thème de l'atelier, faire des expériences conjointes sur le terrain à l'aide de différents techniques pour en vérifier la comparabilité et la fiabilité et définir les grandes lacunes et les besoins urgents de recherche.

Keynote Presentations

Physics: The physical environment and its effects on primary production processes. E. E. Carmack, Institute of Ocean Sciences.

Chemistry: Nutrient dynamics and primary production across frontal systems. P. J. Harrison, University of British Columbia.

Biology: biological responses to physical forcing, with emphasis on photoinhibition and light/shade adaptation. P. J. Neale, University of California, Berkeley.

Modelling: Modelling the interaction between physical and biological processes. J. J. Cullen, Dalhousie University.

Experimental Program

Group I: Physics; experimental leaders: B. C. Kenney and E. C. Carmack. Group II: Nutrient stimulation; P. A. Chambers and J. G. Stockner. Group III: Light/shade adaptation; R. D. Robarts and P. J. Neale. Group IV: *P/R* quotients; M. L. Bothwell and T. Bott.

Experimental Sites

Redberry Lake (110 km northeast of Saskatoon, TDS $\sim 14 \text{ g}\cdot\text{L}^{-1}$, typical June primary production $\sim 30 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$); South Saskatchewan River (oligotrophic above Saskatoon, N and P gradients downstream for $\sim 50 \text{ km}$); laboratory (well-equipped modern laboratory at National Hydrology Research Institute).

General Information

Participation in GAP 6 will be limited to 80 persons. Contributed papers will be limited to poster presentations (maximum $100 \times 200 \text{ cm}$). Accommodation will be on the University of Saskatchewan campus ($\sim \$25\text{--}31 \text{ Cdn/night}$). Workshop fees will be $\$250 \text{ Cdn}$.

The 1993 ASLO meeting will be held in Edmonton, Alberta, from 30 May to 3 June. Following the post-ASLO field trips, a bus will take GAP participants to Saskatoon (5 hours) on 6 June for a $\$50$ fee.

Persons wishing to participate in GAP 6 are asked to contact the Chair before **15 April 1993**: Richard Robarts, GAP 6 Chair, National Hydrology Research Institute, Environment Canada, 11 Innovation Blvd., Saskatoon, Sask. S7N 3H5, Canada. Telephone: (306) 975-6047; Fax: (306) 975-5143.

Présentation clés

Physique : Le milieu physique et ses effets sur les processus de production primaire. E. C. Carmack, Institut des sciences de la mer.

Chimie : Dynamique des matières nutritives et production primaire dans les systèmes frontaux. P. J. Harrison, Université de la Colombie-Britannique.

Biologie : Réponses biologiques au forçage physique, avec accent sur la photoinhibition et l'adaptation à la lumière et à l'ombre. P. J. Neale, Université de la Californie, Berkeley.

Modélisation : Modélisation de l'interaction entre les processus physiques et biologiques. J. J. Cullen, Université Dalhousie.

Programme expérimental

Group I : Physique; directeurs d'expérience : B. C. Kenney et E. C. Carmack. Group II : Stimulation de nutriments; P. A. Chambers et J. G. Stocker. Groupe III : Adaptation à la lumière/ombre; R. D. Robarts et P. J. Neale. Groupe IV : Quotients *P/R*; M. L. Bothwell et T. Bott.

Sites expérimentaux

Lac Redberry (110 km au nord-ouest de Saskatoon, MTD $\sim 14 \text{ g}\cdot\text{L}^{-1}$, production primaire type de juin $\sim 30 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$); rivière Saskatchewan Sud (oligotrophe en amont de Saskatoon, gradients de N et P en aval sur $\sim 50 \text{ km}$); laboratoire moderne et bien équipé à l'Institut national de recherche en hydrologie.

Information générale

La participation au GPA 6 sera limitée à 80 personnes. Les communications seront limitées à des affiches ($100 \times 200 \text{ cm}$ maximum). L'hébergement se fera sur le campus de l'Université de la Saskatchewan ($\sim 25\text{--}31 \$ \text{ Cdn/nu}$). Les frais d'inscription aux ateliers seront d'environ $250 \$$.

Le réunion de 1993 de l'ASLO aura lieu à Edmonton, en Alberta, du 30 mai au 3 juin. Après les excursions qui suivront l'ASLO, un autobus transportera les participants du GPA à Saskatoon (5 heures) le 6 juin à un $50 \$ \text{ Cdn}$ prix.

Les personnes désireuses de participer au GPA 6 sont priées de communiquer avec le Président avant **le 15 avril 1993** : Richard Robarts, président du GPA 6, Institut national de recherche en hydrologie, 11, boul. Innovation, Saskatoon (Saskatchewan) S7N 3H5, Canada. Téléphone : (306) 975-6047; télécopieur : (306) 975-5143.

Fee, E. J., and R. E. Hecky. Introduction to the Northwest Ontario Lake Size Series (NOLSS)	2434-2444
Fee, E. J., J. A. Shearer, E. R. DeBruyn, and E. U. Schindler. Effects of lake size on phytoplankton photosynthesis	2445-2459
Comeau, M., and G. Y. Conan. Morphometry and gonad maturity of male snow crab, <i>Chionoecetes opilio</i>	2460-2468
Cowey, C. B., and C. Y. Cho. Failure of dietary putrescine to enhance the growth of rainbow trout (<i>Oncorhynchus mykiss</i>)	2469-2473
Nelson, J. A., and J. J. Magnuson. Metabolic stores of yellow perch (<i>Perca flavescens</i>): comparison of populations from an acidic, dystrophic lake and circumneutral, mesotrophic lakes	2474-2482
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Rowan, D. J., J. Kalff, and J. B. Rasmussen. Estimating the mud deposition boundary depth in lakes from wave theory	2490-2497
Schindler, D. E. Nutrient regeneration by sockeye salmon (<i>Oncorhynchus nerka</i>) fry and subsequent effects on zooplankton and phytoplankton	2498-2506
Havens, K. E. Acidification effects on the algal-zooplankton interface	2507-2514
Lo, N. C. H., I. D. Jacobson, and J. L. Squire. Indices of relative abundance from fish spotter data based on delta-lognormal models	2515-2526
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